Pathogen concentration integrated molecular analysis for SMARTDIAGNOS: the next generation sepsis diagnosis

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Abstracts

8th International Congress "Sepsis and Multiorgan Dysfunction"

Weimar Sepsis Update 2017– Facing the Challenges

September 6–8, 2017
Weimar

Organizer
H. Gerlach
T. Welte
F. M. Brunkhorst

Guest Editors
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German Sepsis Society (DSG)
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Dear colleagues,

During the last two decades, multiple treatment modalities have been tested in the attempt to improve clinical outcomes from sepsis. During this period we have consistently observed initially promising results that have gradually faded in subsequent large clinical trials. Researchers blame this irreproducibility on slow patient accrual (selection bias), the Hawthorn effect (knowing you are being watched changes your practice), beta-type errors (false positive), publication bias and other factors. In a recent publication (Mebazaa et al. Journal of Intensive Care (2016) 4:24) the authors stated, that the major reasons for lack of survival in recent sepsis trials are over-estimated treatment effects, suboptimal pre-clinical models, incorrect treatment targets, and heterogeneity of definitions and patients. Priorities for future sepsis clinical trials are among others, identification of responders to treatment, networks of sepsis investigators experienced in clinical trial conduct, application of the recent S-3 definitions for sepsis and septic shock, targeted clinical trials in relatively homogeneous groups of patients, and pre-specified covariate adjustment of the primary endpoint (heterogeneity) and exclusion of low-risk patients.

The German Sepsis Society (DSG) will address these questions at its 8th Weimar Sepsis Congress entitled ''Facing the Challenges''. Why Weimar? Due to its unique features, the Weimar Sepsis Congress has built up a reputation on a national and international level: leading experts from all over the world with excellent publications in basic and clinical research, no parallel sessions—everything under a single roof—vivid discussions in a smooth and casual atmosphere.

The 8th Weimar Congress—that will once again take place together with the 9th Congress of the German Society of Intensive Nursing Care—will update you on the current state of knowledge about how to improve treatment of patients by adhering to the guidelines but yet in a critical way.

We are looking forward to meeting you!

Kind regards

Prof. Herwig Gerlach
President

Prof. T. Welte
Vice President

Prof. Frank M. Brunkhorst
Managing Director

Contents

Abstract

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Experimental Sepsis Research

006
Infection 2017

Dynamic experimental study of acute kidney failure pathophysiology in abdominal sepsis

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Introduction: Multiple organ failure is a hallmark of abdominal sepsis (AS). Acute kidney dysfunction is a common sequel of sepsis. Sepsis, which is commonly defined as systemic inflammation process resulting from an infection, often results in an abrupt decrease in the kidney's ability to effectively filter the blood. According to the US National Kidney Foundation, sepsis is one of the major causes of acute kidney injury (AKI) and some studies have found that between 32 and 48% of AKI cases were caused by sepsis. It is being suggested that sepsis-induced kidney failure may have a distinct pathophysiology and identity. Particularly complex is AS, which combines pathogenetic mechanisms common to other forms of sepsis but aggravated by involvement of kidneys into inflammatory process and bacterial overload. However, most of the clinical studies of AKI in sepsis are biased due to interference with treatment, etc.
Objectives: The aim of the study is to study the multimodality pathophysiological mechanisms of AKI under AS in dynamics of acute experiment.
Methods: The study is based on acute AS modelled in 38 white Wistar line rats allowing results standardization excluding treatment bias. AS modeled via intraperitoneal injection of *E. coli* O111:B4 endotoxin, (Difco Labs) and 7 lg CFU/ml of live *E. coli* (clinical strain). Sepsis proved by means of clin/lab observations and microbiological study. Renal dysfunction assessed through set of 50+ variables including absolute and conditional diuresis, ions/creatinine metabolism and excretion/clearance markers (ENa+, UK+, EK+, Uc, Pc), filtration/reabsorption values, nitrogen, detox/pH regulation functions (R H2O%, P Na+, FFNa+, EFNa+, RFNa+, TdNa+, ETA, NH3, CH, EH+, etc). Data collected during 6, 24, 48, and 72 h of AS modeling. Bioethical principles strictly obeyed (approval of the local Bioethics Committee #5 /5.10.2017). Statistica 7.0 (StatSoft Inc) software used for analysis.
Results: Diuresis and conditional diuresis dropped 4 fold during 6 h with growing tendencies during 24–72 h. Urine Na+ concentration and excretion grew 10 and 2 fold (6 h) with further drop of these markers. In contrast, K+ concentration and excretion grew significantly during the course of experiment. Creatinine plasma concentration grew 1.9–2.65 times with 8.2–10.6 fold decreased urine concentration and clearance. Urine filtration rate initially dropped 10.1–12.3 times with further partial compensation of 260.96 ± 18.92 at 72 h of experiment vs. 1436.70 ± 73.38 in control (mkl/min $\times 100$g). H_2O reabsorption rate changed unreliably providing partial compensation mechanism. Maximum protein excretion rate observed during 24 h.
Conclusions: Received data confirms existence of two physiological periods of AKI in AS. The first one is characterized by stress-related influence, compensatory hyper activation of adaptive mechanisms. Second phase is characterized by exhaustion of compensation, dysadaptation and kidney dysfunction.

007
Infection 2017

Fast simultaneous assessment of renal and liver function using polymethine dyes in animal models of chronic and acute organ injury

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Introduction: (Multi-)organ dysfunction after systemic infection defines sepsis and reflects one of the most common causes of death on intensive care units worldwide. Obvious lack of treatment options promotes extensive translational research in rodent models in which a reliable assessment of organ dysfunction is critical. Timely diagnosis of impaired organ function is crucial in this context. Particularly kidney and liver dysfunction are of outstanding importance, both leading to subsequent remote organ impairment due to accumulation of endo- and xenobiotic substances. However, diagnostic tools for sensitive and fast detection of organ dysfunction for kidney or liver failure in daily clinical practice are still scarce. At present, most applied biomarkers are static plasma or urine parameters such as bilirubin in case of liver dysfunction or creatinine and urea for kidney failure. These parameters, however, often lack sensitivity and specificity. Sensitive, fast and simultaneous assessment of excretory liver and kidney function is still an unmet need in experimental stress models as well as in critical care.
Objectives: Aim of the study was to characterize polymethin dyes which can be applied for evaluation of hepatic and renal dysfunction, based on their physico-chemical properties and their elimination capacity in vivo.
Methods: Plasma disappearance and elimination rate measurements of simultaneous injected fluorescent dyes DY-780 (hepato-biliary elimination) and DY-654 (renal elimination) using catheter techniques and (confocal) intravital microscopy in animals subjected to different organ injuries: polymicrobial sepsis by peritoneal contamination and infection, ischemia- reperfusion injury (70% liver tissue or bilateral clamping of kidney arteries) and glycerol-induced acute kidney injury caused by intramuscular glycerol injection. Further assessment of organ function using clinical parameters (transaminases, bilirubin, urea, creatinine) and mass spectrometry to assess conjugation of bile acids.
Results: DY-780 and DY-654 have organ specific and determined elimination routes in healthy and diseased animals. They can be measured simultaneously using (near)infra-red imaging techniques and spectrophotometry. DY-780 and DY-654 plasma-disappearance rate indicate hepatic or kidney dysfunction respectively in different animal models with superior sensitivity than conventional biomarkers. Greatest impact on liver function was found in animals with polymicrobial sepsis whereas glomerular filtration damage due to glycerol-induced kidney injury had strongest impact on DY-654 elimination.
Conclusions: Hepatic elimination and renal filtration can be assessed in rodents measuring DY-780 and DY-654 plasma-disappearance rate. Conventional biomarkers correlate with organ dysfunction assessed by polymethin dyes. Polymethine-dye clearance thereby allows for the first time point-of-care assessment of both organ functions simultaneously.

012
Infection 2017

Experimental study of patients' status severity scoring systems use in abdominal sepsis

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Introduction: Abdominal sepsis (AS) is almost the heaviest example of sepsis associated with a large medical and economic burden. Sepsis understanding switched from 'bacteremia' and even SIRS associated with infection to a multi organ dysfunction caused by an up- and down-regulated host response to infection. Severity of status assessment is currently standardized in septic patients using multimodal scoring systems that can more accurately and objectively determine the status, correct treatment policies, the surgical tactics and outcome. There are many rating scales and systems for AS, among them SOFA, qSOFA, MIP, APACHE (I–III), PSS, ASA, which are often not sufficiently consistent and comprehensive. Their scope is constantly expanding and currently covers the diagnosis, prognosis, treatment, and surveillance for many diseases and injuries.
Objectives: The aim of the study is to determine the feasibility of using the most common scoring systems in comparative aspects in acute standardized experimental conditions.
Methods: Prognostic evaluation of the effectiveness of scoring pathologic conditions was studied by simulating an acute peritonitis in 19 inbreed rabbits weighing 5.39 ± 0.47 kg according to self-developed experimental technique by introducing standardized mixture of museum strains of pathogenic and conditionally pathogenic microorganisms into peritoneal cavity with the addition of adjuvants, which allowed simulating different degrees of severity of the pathological process and progress. The functional state of the organism assessed via heart rate (HR), respiratory rate (RR), arterial blood oxygenation indices (PaO2), arterial blood pH, ionogramm indicators, hematocrit, creatinine, peripheral blood WBC count and formula, morphologic evaluation of the pathological process development and severity. The study approved by the bioethics commission.
Results: In 6 (31.6%) animals modeled local limited peritonitis (1 group), 7 (36.8%)—diffuse (2 group), and in 6 (31.6%)—general peritonitis (3rd group). 12 and 24 h after initiation of a pathological process defined physiological and laboratory parameters and calculated severity for different systems (APACHE, APACHE II, MIP). The calculated results are presented in Figs. 1–3. In group 1 APACHE and MIP indices in contrast to APACHE II decreased after 24 h. Predicted mortality among animals of the group 1 was to be 0% (MIP) or 0–5% (APACHE II and APACHE), 2–29% (MIP) and 5–25% (APACHE II and APACHE). Mortality in group 3 was predicted to be 100% (MIP) and 25–100% (APACHE II and APACHE). Actual mortality was in the group 1—0% in 2—28.6%, the 3rd—83.3%.
Conclusions: The use of scoring systems as prognostic tool is expedient for practical application in clinical settings. In order to increase the probability of forecasting and optimization of treatment strategy it is rational to combine different evaluation scoring systems.

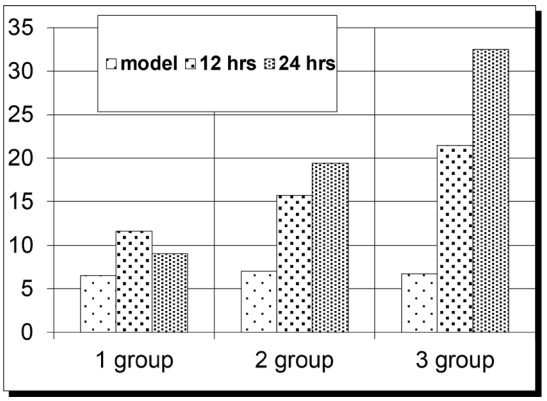

Fig. 1. APACHE values changes in experiment

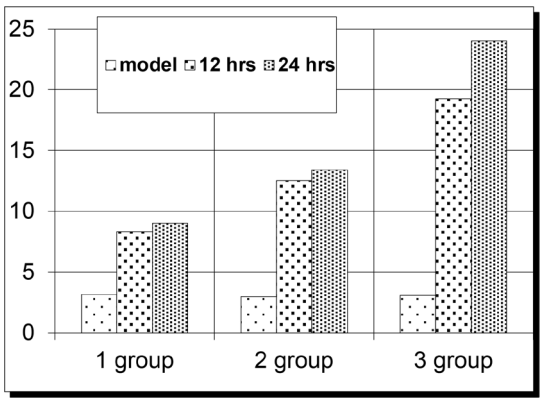

Fig. 2. APACHE II values changes in experiment

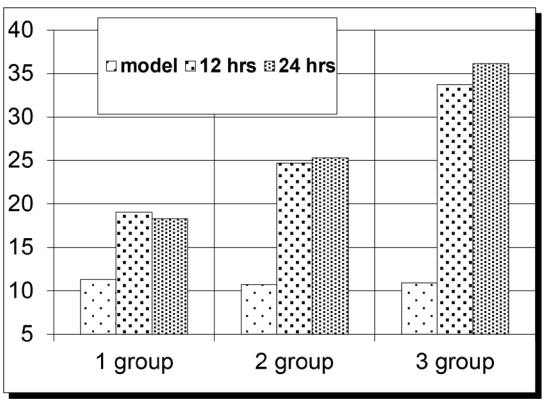

Fig. 3. MIP values changes in experiment

023
Infection 2017

Comparative analysis of infected and uninfected biliary peritonitis

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Introduction: Acute biliary peritonitis is a common cause of abdominal sepsis. While understanding of sepsis require participation of microbiota and microbial toxins, biliary peritonitis adds another peculiarities into sepsis pathogenesis through involvement of bile, liver and kidney dysfunction.
Objectives: The aim of the study was to find the possible influence of biliary peritonitis on kidney function.
Methods: In experimental studies 110 white mature male rats weighing 180–200 g underwent a comparative analysis of infected and uninfected bile peritonitis. Bioethics were strictly obeyed.
Results: In case of non-infected bile peritonitis was negative correlation of distal transport of sodium ions relative reabsorption of water and glomerular filtration in contrast to infected peritonitis in which these relationships are positive, which indicates the difference of mechanisms of development of renal impairment. However, there was general laws, the manifestation of which are the positive correlation of relative water reabsorption from the glomerular filtration, absolute proximal reabsorption of sodium ions. Under the condition of an infected biliary peritonitis, in contrast to uninfected develops the syndrome of "loss" ions of sodium, proteinuria, the decline in the excretion of acids and ammonia from urine. Subject to biliary peritonitis oxidative modification of proteins ends with the formation of acidic groups of proteins, which is evidence that impaired balance of pro - and antioxidant system, the changes are most significant in the case of an infected biliary peritonitis. This increases the oxidative modification of proteins in 1.3 times in kidney and liver of rats according to the ratio R/B, which increases in the proximal 1.5 times, 0.7 times distal parts of the nephron, the collecting tubules of the renal papilla and in the cytoplasm of hepatocytes. For the infected bile peritonitis found a statistically significant ($p < 0.001$) the growth rate. The sequence distribution of the degree of damage of the investigated structures of the liver and kidney indicates the greatest sensitivity determine the degree protein ratio R/B in the proximal nephron and in the cytoplasm of hepatocytes, compared with intact animals, and the infected bile peritonitis found a similar pattern in relation to the uninfected, which confirms the role of infectious factor in the development of reactions of alteration. In the experiment the optical density of plasma venous blood, provided the uninfected biliary peritonitis does not differ significantly from the benchmarks, however, provided the infected peritonitis parameters increased statistically significantly ($p < 0.01$) rise.
Conclusions: The results of the research showed differences of uninfected and infected biliary peritonitis, which has important practical significance to prevent the development of septic infection.

024
Infection 2017

Protein kinase C as target to resolute excretory liver failure in sepsis

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Introduction: Excretory dysfunction of the liver is an early and frequent event in sepsis. Different PAMPs but especially cytokines as well as chemokines from circulating or local recruited as well as inflammatory cells affect hepatic integrity. This sometimes only subclinical excretory dysfunction is strongly associated with survival of the host, both in rodent models and the clinical context, reflecting a critical condition with lacking effective therapies. The protein kinase C isoforms are involved in membrane shaping processes, cytoskeletal remodeling but also cellular polarization, metabolism and many other essential cellular processes. In hepatocytes protein kinase C isoforms are recently described to be essential for the anchoring and trafficking of transporters involved in the basolateral uptake and canalicular excretion of endo- and xenobiotics.
Objectives: The present study investigates for the first time the clinical relevance of Gö6850, a well-established pan-PKC inhibitor for the treatment of liver dysfunction in a murine model of polymicrobial, abdominal sepsis.
Methods: In accordance with Thuringian animal welfare association sepsis was induced applying the model of peritoneal contamination and infection (PCI) which lead in this case to a systemic infection dominated by *Escherichia coli*. Sham animals were injected intraperitoneal with sterile saline. Further supportive volume and antibiotic therapy for 7 days after onset of the infection was applied to all groups. Different treatment regimens are applied during the first 5 days of the infection, in which phase liver failure determines outcome. Animals were observed for a total period of 22 days after infection.
Results: No signs of illness were observed in any treatment or vehicle control groups in sham. An increased survival during every time-point of observation resulting in a survival rate of 40% (PCI, treatment group) versus 18% (PCI, vehicle group) was observed for low doses. Higher doses are only translated in an increased survival within the first days but fail to improve 22 days survival significantly. In a second set of experiments liver tissue was harvested and analyzed for 21 unconjugated and conjugated bile acids (BA) using mass spectrometry. An accumulation of unconjugated BA was found in PCI animals ($n = 6$) after 24 and 72 h but not in sham vehicle or any sham Gö6850 groups. Treating infected animals with higher doses of Gö6850 lead to no elevation of BA after 24 or 72 h. Treatments with low doses Gö6850 per animal once daily lead to an initial increase of BA after 24 but dropped to baseline after 72 h.
Conclusions: Treatment of septic cholestasis with Gö6850 resolute excretory dysfunction in a dose- and time-dependent manner and increases survival from systemic infections significantly in a murine model of sepsis, emphasizing the outstanding importance of protein kinases in the development of liver failure during infection and gives for first time an effective treatment targeting cholestasis.

029
Infection 2017

Underlying pathogen determines characteristics of liver failure in sepsis

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Introduction: The development of organ dysfunction discriminates uncomplicated infection from sepsis. The current concept assumes that organ dysfunction occurs as a result of inadequate host response independent of the underlying pathogen. Life-threatening infections are treated primarily as bacterial infections, resulting in a depletion of microbiota-derived metabolites, enhancing pathogen susceptibility, impairing immune homeostasis and promoting fungal infection. For different gut-derived pathogens the liver is described as a first target organ, where systemic infection establishes.
Objectives: This study aims to determine whether the hepatic host response to bacterial and fungal infections differs with a focus on hepatic metabolism and liver function.
Methods: Murine peritoneal bacterial contamination and infection (PCI) as well as intraperitoneal and systemic *C. albicans* infection models were compared 6 and 24 h post infection (p. i.) to sham controls. Liver tissue was analyzed regarding protein-protein-interaction networks using omic-strategies. Specific readouts such as intravital microscopy and immunofluorescence microscopy were used to analyze specific aspects of pathophysiology such as cholestasis.
Results: Transcriptomics revealed similar alterations of hepatic metabolism in all three infection groups; the degree of regulation however differed between the infection models and time point. Most notable, besides the immune response, was the downregulation of lipid catabolism and bile acid (BA) synthesis already after 6 h in all groups. Alterations in lipid catabolism were characterized by an accumulation of long chain acylcarnitines, while short chain fatty acids showed a more distinct behavior between groups. Defective beta-oxidation affected the energy metabolism as early as 6 h p. i. as indicated by reduced hepatic NADH. Intrahepatic BA revealed different conjugation-patterns in PCI vs. Candida infection. While PCI led to accumulation of unconjugated BA, *C. albicans* infection caused accumulation of conjugated BA independent of the route of infection. Hepatic dye clearance and transporter expression revealed reduced uptake in fungal infections vs. defects in secretion following bacterial PCI.
Conclusions: While many aspects of the host response to bacterial and fungal infections appeared to be conserved, molecular phenotypes of lipid accumulation and cholestasis allow differentiation between pathogen and route of infection already at early stages, with possible diagnostic applications. The consequences for outcome and therapeutic approaches yet need to be determined.

030
Infection 2017

Targeting cholesterol signaling to reduce pneumolysin-induced cellular injury in hepatocytes and alveolar macrophages

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Introduction: Sepsis reflects a frequent clinical condition characterized by organ failure including hepatic or pulmonary dysfunction. Underlying infections, such as pneumonia, are often caused by *S. pneumonia*, a bacterium producing the cholesterol-dependent cytolysin pneumolysin (PLY). PLY induces cell lysis and thereby triggers barrier failure allowing bacterial expansion all within and beyond the lung. The body reacts to PLY via a hepatic remote organ response: An increase in sterol signaling and particular an upregulated cholesterol-biosynthesis of unknown signaling origin and biological function.
Objectives: Here we investigated the role of the adaptive hepatic sterol-response to PLY and whether cholesterol, formulated as cholesterol-containing particles (CCP), can reduce PLY induced cellular toxicity by neutralizing.
Methods: Alveolar macrophages (AM) were lavaged from lungs of FVB/N mice, maintained in tissue culture for 24 h and afterwards treated with PLY, cholesterol and/or CCPs for 3 and 6 h. Toxicity was evaluated by LDH-release assay. Same procedure was performed with hepatocyte carcinoma cells (HepG2). Also RNA was isolated from treated HepG2 cells to analyze gene expression of specific patterns of sterol-cholesterol-pathway.
Results: Our results indicate that PLY treatment leads to a concentration-dependent toxicity in both cell-types. However AM are more sensitive within the used concentrations. Cholesterol treatment directly before addition of PLY results in both cell-types in decreased PLY-induced cellular toxicity in a concentration-dependent manner. In AM this protective effect is present after 3 h as well as after 6 h of incubation with PLY whereas in HepG2 cells the protective effects are time-dependent. To employ cholesterol in vivo CCPs with two diameters ($d < 200$ nm and $d > 1$ µm) were prepared based on various biodegradable polymers. Treatment with CCPs (but not cholesterol-free particles) reduces PLY-mediated cell toxicity in both cell-types in a time-independent manner. Due to previous mouse studies, where *S. pneumonia* lung infection leads to a hepatic sterol response, genes encoding proteins of sterol-biosynthesis (HMGCR, SREBP-1, and SREBP-2) are therefore up-regulated in HepG2 cells when exposed to PLY. Further the sterol response was cholesterol-feedback sensitive, thus supplementation of cholesterol (free and in particles) lowered the sterol response significantly.
Conclusions: These results demonstrate the protective effects of cholesterol and CCPs in the light of PLY induced cell damage and death. Also PLY can directly act on hepatocytes, where it triggers the activation of sterol-synthesis but not circumventing intrinsic negative feedback-loops. The adaptive hepatic sterol signaling might therefore be seen as response to counteract caspase-mediated pro-apoptotic and inflammatory signaling as well as intrinsic mechanism to inhibit PLY. Our data further suggest, that the mechanism of cholesterol-dependent protection counteracting PLY differ between AM and hepatocytes.

032
Infection 2017

Multispectral optoacoustic tomography to assess liver and kidney function in Sepsis by measuring dye clearance

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Introduction: Sepsis is a life threatening organ dysfunction caused by a dysregulated host response to infection that is associated with hospital mortality rates exceeding 40%. Impaired organ function, induced directly by pathogens or by the immune system (immunopathology) determines survival of critically ill septic patients. As sepsis-induced liver and kidney dysfunction may be unrecognized, diagnostic methods for early and effective detection of sepsis-associated organ dysfunction and damage are essential to initiate potentially life-saving therapy and/or adequate therapeutic adjustments. Multispectral optoacoustic tomography (MSOT) is a recently developed sectional imaging modality that allows detection of infrared absorbent molecules in a non-invasive manner.
Objectives: It has been shown that Indocyanin green (ICG) and IRDye800CW (IRD) plasma clearance can be measured by MSOT in mice. However, data analysis is currently done in a non-systematic manner, thus leaving the effect of pathological conditions on ICG and IRD unclear. In this study we aim to develop a systematic image analysis strategy to characterize spatio-temporal dye-clearance in health and disease as measured by MSOT.
Methods: Repetitive measurement of organ function by MSOT in the murine peritoneal contamination and infection model of sepsis were performed for 28 days. Liver and kidney function was assessed by using i.v. injection of ICG and IRD, respectively, and MSOT images were obtained from the organ areas every 15 s for 20 min. The raw MSOT data were backprojected and unmixed, resulting in MSOT channels specific for water, oxy- and deoxyhemoglobin, ICG and IRD. Our custom-written software extracted kinetic information of ICG and IRD clearance from the liver and the kidneys, respectively. The software used the spinal cord, the aorta and the vena cava as reference points in order to standardize the spatial coordinate systems temporally, as well as between animals. The typical kinetic behavior of ICG and IRD were extracted from the MSOT images using regions of interest (ROI) that covered the corresponding organs. A gold standard was provided by the manual selection of 2000 and 700 ROI in the liver and kidney areas, respectively, in a typical image set.
Results: MSOT together with our analysis software allows the simultaneous assessment of liver and kidney function. We find that the plasma disappearance rate (PDR) is lower in PCI animals compared to sham for both ICG and IRD. We examine the correlation between PDR and survival rate in various PCI animals. Finally, we are aiming to automatically identify and characterize the anatomical structures based on their pharmacokinetic behavior.
Conclusions: MSOT is a very promising technique to detect early sepsis-associated liver and kidney impairment by close monitoring of the organs' perfusion, oxygenation and function during disease progression in a longitudinal manner.

033
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Investigation of the Role of Hepatic Fam 134 isoforms in Autophagy

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Introduction: Autophagy is an important regulator of cellular responses during sepsis and plays a pivotal role in cell repair, development, differentiation, as well as immunologic defense. Macroautophagy (henceforth referred to as autophagy), consists of the formation of a double-membrane vesicle known as the autophagosome. The autophagic cascade is mediated by autophagy-related (Atg) proteins and receptors. The autophagy-related protein 8 (Atg8) Ubiquitin-like proteins include the LC3 and GABARAP subfamilies, to which FAM134A, FAM134B and FAM134C can bind to facilitate ER degradation. Downregulation of FAM134 causes expansion of the ER, while overexpression causes ER fragmentation and lysosomal degradation.
Objectives: Here we investigate the role of Fam134 isoforms in autophagosome maturation and in hepatic ER stress by analyzing the levels of autophagic markers in primary hepatocytes stimulated with tunicamycin, LPS, and rapamycin.
Methods: As an ex-vivo sepsis model, primary hepatocytes were isolated from both knockout and wild-type mice for each isoform, and treated following an equilibration phase of 24 h with tunicamycin, lipopolysaccharide (LPS), and rapamycin for 5 h. Proteins were detected and analyzed by (neoepitope) specific immunoblotting, while expression analyses were performed by quantitative polymerase chain reaction and normalized using the Pfaffl method.
Results: The Fam134B +/+ cells demonstrated a marked increase in the Beclin-1 and XBP1 expression ($n = 4$, $n = 6$) when treated with LPS, tunicamycin, and rapamycin. Intriguingly, Fam134B−/− showed a significant increase in p62/SQSTM1 expression compared to Fam134B +/+, indicating that the mutation may have caused impairment in autophagy that directly influences p62. Similarly, Fam134B−/− showed a stronger increase in LC3B II than Fam134B+/+ upon induction of autophagy. Interestingly, a gender-based difference was observed in the induction of autophagic marker expression. For example, the Fam134C −/− females showed an unexpected increase in Beclin-1 and XBP1 protein levels as compared to the Fam134C+/+ females. Conversely, the Fam134C +/+ males showed higher levels of Beclin-1 and XBP1 protein as expected. Cells stimulated with tunicamycin showed a significant ($p < 0.05$) increase in mRNA levels of BiP and ATF4 haphazard of the mutation. Furthermore, XBP1 splicing was observed in tunicamycin stimulated cells, indicating that tunicamycin plays a significant role in the unfolded protein response (UPR) machinery in both wild-type and knockout cells
Conclusions: LPS, tunicamycin, and rapamycin induced the expression of different markers involved in autophagosome formation and maturation. Tunicamycin induced an increase in the mRNA levels of BiP, ATF4, and spliced XBP1, which play a vital role in hepatic ER stress. En masse, these findings reveal insights into the processes underlying the induction of autophagy in sepsis, and how these pathways could be exploited to design novel therapeutic strategies for sepsis.

037
Infection 2017

Matrix metalloproteinase 14 induces Tie2 shedding in experimental sepsis and human disease

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Introduction: Tie2 is an endothelial tyrosine kinase receptor that controls vascular quiescence and its response to injury. Besides its deactivation through antagonistic ligands, Tie2 expression per se dramatically decreases during sepsis. Experimental reduction of Tie2 recapitulates cardinal features of septic vasculature and increases the host's susceptibility to the disease.
Objectives: Here, we investigate the molecular mechanism of Tie2 protein suppression in sepsis in order to develop effective therapeutic strategies.
Methods: In vitro (stimulated endothelial cells) and in vivo (C57/Bl6 murine cecal ligation and puncture and LPS) models of sepsis plus retrospective analysis of human septic shock samples ($n = 57$).
Results: We found that Tie2 was rapidly suppressed both in vitro and in vivo up to 79.7% within 8 h and 56.6% within 4 h, respectively. We observed an increase in the extracellular (so-called soluble) Tie2 fragment (75 kDa) cross-species and in different disease models (CLP and LPS) in the supernatant of challenged endothelial cells (ECs), the circulation of septic mice (1.07 vs. 1.45 ng/mL, $p = 0.001$) and the circulation of septic humans (12.29 vs. 18.77 ng/mL, $p < 0.0001$). In vitro, we identified MMP14-dependent Tie2 ectodomain shedding as putative underlying mechanism that was protected both after genetic MMP14 knockdown and pharmacological MMP inhibition. Consistently, supplementation of recombinant human MMP14 to ECs increased spontaneous Tie2 shedding. Moreover, experimental reduction in MMP14 increased protective Tie2 downstream signaling (pAKT) and improved endothelial barrier function (transendothelial electrical resistance).
Conclusions: Together, these data indicate that acute changes in Tie2 expression might be regulated via ectodomain shedding in an MMP14-dependent manner. Therapeutic protection of Tie2 expression might represent a central way to maintain vascular quiescence.

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Infection 2017

Flow-regulated GATA3 Expression Controls Tie2 Transcription in Sepsis

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Introduction: Tie2 is a barrier-protective tyrosine kinase receptor that is almost exclusively expressed by endothelial cells (ECs) and maintains vascular barrier function and endothelial quiescence. In systemic inflammation, Tie2 signaling is altered by de-activation of Tie2 by its antagonistic ligand (termed Angiopoietin-2) and by Tie2 ectodomain shedding. Recently, we have additionally observed an acute drop in Tie2 transcription upon various models of murine critical illnesses.
Objectives: We looked for a common chief mechanism of negative Tie2 mRNA regulation in sepsis leading to a capillary leakage and multiple organ dysfunction.
Methods: Basic molecular biology methods (qPCR, WB, siRNA, plasmid transfection) for in vitro (endothelial cells in a microfluidic chamber to apply flow) analysis and in vivo murine sepsis models (cecal ligation and puncture (CLP) and endotoxemia (LPS)). Additionally, qPCR for Tie2 and GATA3 from post mortem kidney biopsies from septic AKI (acute kidney injury) patients ($n = 14$).
Results: In murine experimental sepsis, we found a rapid and profound suppression of Tie2 mRNA by >90% in less than 4 h in different vascular beds. A common denominator of clinical sepsis is shock leading to microvascular hypoperfusion that led us to investigate flow-regulated endothelial gene responses that could control Tie2 mRNA. In in vitro flow experiments (to model hypotensive shock) we identified GATA binding protein (GATA) 3 as a potential regulator of Tie2 transcription. GATA3 was also significantly reduced in different murine sepsis models (CLP and LPS). Of note, flow dependent GATA3 induction was required for ECs to increase Tie2 transcription but GATA3 alone was not sufficient to boost Tie2 transcription. Importantly, in post mortal human biopsies from septic individuals Tie2 and GATA3 were also reduced and significantly correlated with each other highlighting the putative translational relevance of this finding.
Conclusions: Given that we observed a drop in Tie2 mRNA in various models of critical illness we believe that the common driver could be "shock" that leads to reduced microvascular flow thereby negatively affecting flow-dependent gene responses. Our data indicate that transcriptional changes of GATA3 might count responsible for the acute reduction in Tie2 mRNA and vascular destabilization in sepsis.

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Infection 2017

Microbiota of the albino rats colic lumen under abdominal sepsis

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Introduction: Abdominal sepsis (AS) continues to occupy a leading position by the severity of the course, frequency of complications and mortality among acute surgical pathologies. The main sources of infection are microorganisms that persist in the lumen and in biotope of the mucous membrane of the small and large intestine.
Objectives: To study the qualitative and quantitative composition of the microbiota of the contents of the colic lumen of albino rats with AS.
Methods: The experiment was conducted on outbred albino rats (body weight 200–220 g). All animals were quarantined for 10–14 days in vivarium. Ten rats with induced abdominal sepsis were included in the main group, 15 intact animals formed the control. Before the study all animals were examined for possible pathology. In sterile conditions, the abdominal cavity was opened, a portion (1.5–2 cm) of the colon with its contents was taken. A sterile 0.9% sodium chloride solution was added to the contents. The series of ten-fold dilutions with a concentration of the initial mixture of 10–2 to 10–10 were prepared. From each test tube 0.01 ml was applied to appropriate solid nutrient media, which are optimal for microbes.
Results: Bacteroides, Escherichia, Lactobacillus, Proteus, Clostridium, Bifidobacterium, Peptococcus, Staphylococcus are dominant in the colon cavity of experimental animals with AS according to microecological indices (Margalef, Berger-Parker and Simpson indices). Peptostreptococcus, Klebsiella, Enterobacter and yeast-like fungi of the genus Candida belong to the additional microflora. Enterococci, according to their indices, are represented as accidental microflora, although in intact rats they form a dominant microbiota. In experimental animals with AS the population level of colony-forming (viable) Bifidobacteria decreases by 2.06 times, Lactobacilli—by 2.04 times. Also, the population level of Peptostreptococci is reduced by

50.13%. On the background of a decreased number of anaerobic functionally significant bacteria, there is an increase in the role of conditionally pathogenic Bacteroides by 21.04%, Peptococci—by 54.59%, and Clostridia—by 55.64%. In this case, microorganisms that contaminate the cavity of the colon reach a high (from 4.95 ± 0.18 to 5.93 ± 0.27 lg CFU/g) population level. Aerobic microorganisms dominated by 36.22% compared with ones in intact animals, where anaerobic bacteria predominate more than twice.
Conclusions: In the colon cavity of the outbred albino rats with experimental AS severe disorders of microbiota in comparison with intact animals are formed. That results in changes of taxonomic composition and population level of its representatives. According to the quantitative composition of microorganisms of the colon cavity of albino rats with AS they are displayed in the following order: bacteria of genera Escherichia, Bacteroides, Proteus, Clostridium, Lactobacillus, Peptococcus, Bifidobacterium, etc. The quantitative composition of aerobic microorganisms dominated over anaerobic bacteria.

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Infection 2017

Development of a human gut-on-chip model to study macrophage dependent inflammatory response

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Introduction: Under normal conditions the intestinal epithelium forms an essential barrier to prevent translocation of microorganisms, toxins or other potentially harmful molecules to our body. Especially dendritic cells in the intestinal epithelium provide important tolerance signals to intestinal microorganisms which thus do not act as pathogens but appear to modulate different physiological, metabolic and immunological functions of their hosts. Pathophysiological conditions of sepsis however, are typically accompanied with a disruption of the epithelial and endothelial gut barrier function allowing access of pathogens to the circulation and thus to different tissues and organs.
Objectives: In this study we aimed to establish a microfluidically perfused three-dimensional cell culture system of the human gut to elucidate the molecular and cellular mechanisms of inflammation-associated gut barrier breakdown.
Methods: A human gut-on-chip model was developed based on our previously developed MOTiF (multi organ tissue flow) biochips made from polystyrol. The biochips are composed of two chambers separated by a micro-porous membrane. Each chambers is connected to inlet and outlet channels allowing independent perfusion of the individual channels and application of microfluidic shear stress. Human umbilical vein endothelial cells (HUVECs), tissue resident macrophages and intestinal epithelial cells (Caco-2) were assembled on the biochip membrane and grown for 7–14 days.
Results: Within two weeks of perfusion the gut-on-chip models formed self-organized and well-polarized villus- and crypt-like structures that resemble essential morphological characteristics of the human intestine. Integrated human macrophages mimicking dendritic cells specifically respond to bacterial lipopolysaccharide (LPS) challenge depending on the side of stimulation. LPS is well-tolerated at the epithelial side corresponding to the gut lumen without signs of macrophage activation or a significant release of cytokines. However, LPS stimulation at the endothelial side of the gut-on-chip model triggers a pro-inflammatory macrophage activation and the release of cytokines such as TNF, IL-1β, IL-6 and IL-8.
Conclusions: The gut-on-chip model can be used to gain more detailed insight into the interplay between commensal bacteria (*Lactobacilli, E. coli* and *Enterococci*) and the human gut. Furthermore, it will be used to study mechanisms of immune tolerance and host-pathogen interaction. Therefore, infection models of pathogenic *E. coli* as well as Candida albicans are currently established that will be used to explore mechanisms of gut barrier break-down during sepsis.

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Infection 2017

Lung-on-chip model for sepsis research

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Introduction: To date, the majority of investigations in regard to microbial infections of the lung rely on animal models such as rodents. However, lung anatomy and physiology as well as composition of the immune system significantly differs between rodents and men. Therefore, considerable advances have been made in the development of cell culture models as surrogates of human lung tissue. However, human cells commonly fail to maintain differentiation and expression of lung-specific functions, due to i.e. cancerous origin of the cells and static mono-cell culture conditions. A human alveolus-model recreating a reactive tissue-tissue interphase between the vascular endothelium and the airway-facing epithelium is therefore desirable. It could allow a deeper insight into infection mechanisms of pathogens and their persistence in human lung tissue.
Objectives: The aim was to establish a human in vitro alveolus model composed of vascular and epithelial cell structures with integrated macrophages resembling the human alveolus architecture and function. The alveolus model should owe a high barrier integrity of the air-liquid-interphase, that can be maintained for up to 14 days. In addition, expression and localization of functional cell type-specific marker and surfactant production should be similar to human lung tissue. Combining all these characteristics it should be suitable for infection studies of microbial organisms and their pathogenesis.
Methods: MOTiF biochips were seeded with human endothelial cells on the vascular site and with epithelial cells and macrophages on the airway site. This organoid was cultured for up to 14 days with a robust and stable air-liquid interphase (ALI) under dynamic flow conditions. Barrier integrity was proven by transepithelial electrical resistance (TEER) measurements and permeability assays. Expression and localization of cell-type specific markers and functional proteins was proven by immunofluorescence.
Results: Dynamic conditions for maintaining ALI allow a stable barrier with high transepithelial resistance and an intact vascularity. The introduction of macrophages into the model resulted in a significant increase of barrier integrity proven by TEER measurement and permeability tests. In addition, epithelial cells show high expression of tight junction proteins claudin 3 and occludin. Epithelial cells in the alveolus model are able to produce surfactant (proven by staining for surfactant protein A), which is important for an adequate immune response to invading pathogens. Macrophages were detectable for up to 14 days.
Conclusions: We established a functional, biochip-based human in vitro alveolus model that could be suitable for infection studies. Separated airway and vascular chambers allow an infection with a pathogen from the airway site. Thereby inducing a immune response it is possible to observe migration of immune cells from the vascular site into the infected sites.

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Infection 2017

SMARTDIAGNOS-Next generation technology for detection of the pathogens causing sepsis—an EU Horizon 2020 innovation project

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Introduction: Sepsis is a potentially fatal condition arising when the body's response to an infection, damages its own tissues and organs. Sepsis is one of the biggest health issues in the EU and worldwide due to its high incidence, mortality, and economic cost. Early diagnosis is crucial, as every hour of delay in appropriate antimicrobial (AM) therapy increases mortality by 5–10%.
Sepsis diagnosis remains one of the greatest challenges in critical care. Current laboratory (LAB) methods for detection of the pathogens causing sepsis, including blood culture and different nucleic acid based multiplex amplification technologies, are impaired by the significant time-delay of 1–2 days and/or low sensitivity of 30–50%. Hence there is an urgent need to develop new diagnostic tools that can provide more accurate and earlier pathogen detection, so that sepsis patients can be administered with rapid and correct AM treatment.
Objectives: The proposed SMARTDIAGNOS platform will advance sepsis diagnosis by simplifying clinical sample analysis methods and integrating the required numerous steps into a streamlined point-of-care (POC) and a LAB device.
Methods: These objectives will be achieved by combining a number of innovative technologies: (1) 3-dimensional concentration of pathogens to process large amount of raw sample (whole blood); (2) direct PCR in the 3D microstructure to circumvent DNA extraction step; (3) solid phase PCR (SP-PCR) to achieve unlimited multiplexing capability and unprecedented selectivity; (4) supercritical angle fluorescence (SAF) microlens array for enhanced fluorescence detection and precise quantification of sepsis-related pathogens; (5) SP-PCR to identify microRNA (miRNA) as biomarkers for the body's response to infection.
Results: In the first year of the project we have: (1) defined user requirements based on literature; on end-user interviews; expert interviews, and review of existing commercial products; (2) designed and fabricated 3-dimensional structures for concentration of pathogens; (3) developed reactions to detect 5 and 30 of the most relevant sepsis pathogen genus and species, respectively, and 6 of the most relevant AM resistant (AMR) genes by SP-PCR; (4) designed and fabricated arrays of SAF microlenses which increases fluorescence sensitivity by 1–2 orders of magnitude; (5) a pilot study resulted in a potential miRNA profile that could be applied as sepsis biomarkers to stratify between patients having bacterial sepsis and patients having non- infectious SIRS. However these results need to be further validated.
Conclusions: In the expected outcome of the project the SMARTDIAGNOS system will go beyond the state of the art for shorter time (1–3 h), higher sensitivity (95%), higher selectivity (99%), multiplexing capability, and automation. Fast and correct detection of sepsis-related pathogens and their AMR genes will improve patient outcome, shorten intensive care stay and thus reduce health care costs.
Reference: http://www.smartdiagnos.eu
Acknowledgement: Thanks to everyone from the SMARTDIAGNOS consortium.

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Infection 2017

Infection-on-chip: *Staphylococcus aureus* infection in a human liver-on-chip model

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Introduction: *Staphylococcus aureus* (*S. aureus*) is an opportunistic pathogen that can colonize the epithelial surface of many healthy individuals, but is also one of the most frequent causes of sepsis. To invade human tissues *S. aureus* could rely on a wide spectrum of different virulence factors, but possess also the ability to dynamically react to environmental changes and adapt to the intracellular milieu by changing its bacterial phenotype to "small colony variants" (SCVs). SCVs are adapted phenotypes for intracellular persistence in endothelial and epithelial cells. Tissue macrophages of the liver, termed Kupffer cells (KC), are critical regulators of host defense against systemic *S. aureus* infections by sequestering the majority of bacteria from the blood stream.
Objectives: The aim was to establish an infection model of *S. aureus* for the human liver to specifically elucidate to role of macrophage polarisation in an adaptive immune response.
Methods: In a microfluidic human liver-on-chip model we investigate the host–pathogen interaction in the context of a complex liver microenvironment.
Results: We focus on the immune response to the pathogen and analyze how different KC activation stages could influence SCV formation and bacterial persistence during the course of infection. In addition, resolvins and lipoxins as pro-resolving lipid mediators secreted by macrophages are characterized for their potential to ameliorate infection associated tissue damage in the context of an adapted immune response.
Conclusions: Revealing the mechanisms of *S. aureus* which allow bacteria to hide within the host and circumvent the hosts defense system is the prerequisite to develop new therapeutic strategies for treatment of *S. aureus* infection-related sepsis and its long term sequelae.

044
Infection 2017

Hemolysis and glucose metabolism during systemic inflammation: responses to intravenous glucose infusions

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Introduction: Systemic inflammation alters glucose metabolism resulting in early hyperglycemia, temporary euglycemia and finally hypoglycemia (1). Severe disorders of glucose metabolism are associated with an increased risk of death (2, 3). A sufficient blood glucose level is particularly important for the CNS, but even more

important for the erythrocytes. Damage of the red blood cells leads to massive hemolysis- and cell-free hemoglobin appears to be an important predictor of survival in sepsis (4, 5).
Objectives: The impact of intravenous glucose infusion on the alterations in glucose metabolism and the release of cell-free hemoglobin during systemic inflammation were investigated in male Wistar rats.
Methods: Sham control group rats (SHAM) received Ringeŕs solution at a rate of 7 ml/kg h over a total period of 300 min. Systemic inflammation was accomplished by continuous lipopolysaccharide infusion (1 mg LPS in Ringeŕs solution/kg h). Glucose was supplied either moderately or excessively during systemic inflammation. During moderate glucose supply, rats received 1 mg LPS in a 1% solution of glucose/kg h (LPS + GLC1%). During excessive glucose supply, rats received 1 mg LPS in a 3% solution of glucose/kg h (LPS + GLC3%). Systemic and vital parameters (e.g., systemic blood pressure) as well as blood and plasma parameters (e.g., concentrations of glucose, lactate and cell-free hemoglobin; activity of lactate dehydrogenase) were measured hourly.
Results: Continuous infusion of LPS led to an early hyperglycemic pre-shock state followed by a later hypoglycemic shock state. It induced severe functional impairment and tissue injury such as metabolic acidosis, electrolyte disturbances, and massive hemolysis (increases in plasma cell-free hemoglobin). Excessive but not moderate glucose supply increased LPS-related early hyperglycemia. Later hypoglycemia was improved by excessive glucose supply (only slightly by moderate glucose supply). Both moderate and excessive glucose supply reduced hemolysis during systemic inflammation. Excessive but not moderate glucose supply enhanced LPS-induced tissue injury measured as an increase in lactate dehydrogenase activity.
Conclusions: Final hypoglycemia during systemic inflammation can be reduced by intravenous glucose infusion. In accord, excessive glucose supply causes an increase in early hyperglycemia (6). More interestingly, however, is the observation that even moderate glucose supply can reduce the LPS-induced release of cell-free hemoglobin—what is known to be an amplifier of LPS biological activity (7). At present, further studies are underway know, to verify whether the decrease of hemolysis is directly attributable to the changes in blood glucose as the sole source of energy for the erythrocytes or is due to the modulatory role of glucose in the inflammation-related activation of coagulation (8). The latter was identified recently as one possible mechanism to induce hemolysis during systemic inflammation (9).
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046
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Dampening inflammation: Co2+-loaded block copolymer micelles as novel tools to trigger anti-inflammatory macrophage polarization

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Introduction: Sepsis is associated with an overwhelming immune response and pro-inflammatory activation of macrophages (Mph) triggered by pathogen-associated molecular patterns (PAMPs). Mph are important regulators of the immune response and could mediate pro-inflammatory but also anti-inflammatory processes during acute phase infection and tissue regeneration, respectively. During acute phase of sepsis, pro-inflammatory cytokines are abundantly released by pro-inflammatory M1 polarized Mph that contribute to inflammation-associated tissue damage. On the other hand, anti-inflammatory M2 polarized Mph contribute to tissue remodeling and repair after initial acute phase reaction to infection.
Objectives: Cobalt 2+ (Co2+) ions have been described to specifically modulate Mph activation through induction of the regenerative M2 polarization state. Nevertheless, free Co2+ is toxic. We therefore synthesized triblock terpolymer micelles for Mph-specific intracellular delivery of Co2+.
Methods: Co2+ loaded micelles were tested for their ability to modulate polarisation state in human primary macrophages in vitro.
Results: We characterized the micelles with respect to size, effective Co2+ loading and impact on cellular viability. Plain and cobalt-loaded micelles with sizes ranging from 15–30 nm were taken up in a concentration-dependent manner through Mph without detrimentally affecting cell vitality. Micelle uptake was found to be independent from active receptor-mediated endocytosis and likely occurred by fusion with the cellular membrane. Co2+-loaded but not plain micelle uptake by non-activated Mph lead to concentration-dependent release of IL-10 with no detectable secretion of TNF or IL-1b. In LPS-activated Mph Co2+ delivery mediated a significant reduction of pro-inflammatory cytokines (TNF, IL1b, IL-8 and IL-6) and a 200-fold increased release of IL-10 compared to unloaded control vehicles. In addition, dampened inflammation was associated with increased expression of M2 polarization markers CD163 and CD206.
Conclusions: In conclusion, Co2+ delivery to LPS activated Mph by triblock terpolymer micelles prevented a pro-inflammatory immune response by inducing an anti-inflammatory M2 phenotype. Co2+-loaded micelles might represent a novel tool to specifically induce M2-Mph polarization also in in vitro tissue cultures. In addition, triblock terpolymer micelles might also be an interesting delivery vehicle for Co2+ to prevent macrophage-dependent tissue damage in vivo.

047
Infection 2017

Macrophages and monocytes as regulators of tissue damage and repair in a human liver-on-chip model

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Introduction: Tissue resident macrophages are important mediators of inflammation. The liver harbors ~80% of all body macrophages which underscores the central immunological role of this organ, especially during sepsis. In adapting their phenotype, Kupffer cells cell can either contribute to inflammatory progress (M1-polarization) or trigger tissue-regeneration and -repair (M2-polarization). This switch is critically regulated via cytokine signaling and mainly driven by circulating blood cells, like monocytes.

Objectives: Based on our recently established human liver-on-chip model we investigated the hepatic dysregulation in respect to macrophage polarization and elucidated the role of circulating monocytes during this inflammatory process. Further, we identified possible regulators of the macrophage polarization switch.
Methods: The microfluidically perfused liver-on-chip model, which includes all major cell types of the liver was challenged with lipopolysaccharide (LPS) to induce inflammation. Pro-inflammatory cytokines (IL-6, TNF and IL1β) as well as anti-inflammatory cytokines were measured in the vascular compartment. Hepatic damage and function was assessed through measurement of clinically relevant parameters. Furthermore, immunofluorescence microscopy as well as celltype-dependent markers were used to determine macrophage polarization state.
Results: Challenge of the liver-on-chip model with LPS led to a pro-inflammatory phenotype of the tissue resident macrophages together with an upregulation of CD197 and HiF-1α, representing an M1-like phenotype. Integration of circulating monocytes prevented hepatic damage. This was most likely associated with an M2-like phenotypic transition of tissue resident macrophages and a higher release of the anti-inflammatory cytokine IL-10.
Conclusions: Our study demonstrates the critical role of tissue-resident macrophages and invading monocytes during hepatic inflammation in a complex microenvironment. Furthermore, it was shown that macrophage polarization critically determines tissue damage and as well as tissue-repair in a human liver-on-chip model.

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Infection 2017

CAAP48—a new sepsis biomarker that has an active role in the pathophysiology of sepsis

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Introduction: Sepsis is a life threatening condition and the leading cause of death in intensive care units. For significant reduction of sepsis mortality a very early diagnosis and identification of high-risk patients as well as a fast and targeted therapy is mandatory. Therefore, identification of reliable biomarkers, facilitating sepsis diagnosis, is still necessary.
Objectives: Using mass spectrometry we identified a C-terminal fragment of alpha-1-antitrypsin, designated CAAP48 (C-terminal peptide of alpha-1-antitrypsin with a mass of 4.8 kDa), that we found to be elevated in septic patients. Furthermore, we could show that this proteolytic fragment acts pro-inflammatory and influences the homeostasis of immune cells as well as endothelial cells and hepatocytes.
Methods: CAAP48 concentrations were determined in plasma samples of sepsis patients and in the time course of sepsis by LC-MS/MS. To examine the effect of CAAP48 on immune cells fresh isolated PMN/monocytes were incubated with different concentrations of synthetic CAAP48 and several control peptides. The expression of activation markers was analyzed by flow cytometry. The chemotactic response was examined using disposable Boyden chambers. The viability was determined based on Annexin V/propidium iodide staining. To analyze the pathophysiological function of CAAP48 on human endothelial cells and hepatocytes we used a microfluidic-perfused in vitro organoid model of the human liver sinusoid that allows studies under physiological conditions. Immunofluorescence staining was done with antibodies against: MRP2, VE-cadherin and F-actin. Secreted cytokines were measured in collected supernatants using a commercially available cytometric bead array.
Results: CAAP48 was observed to be present in 3-4 fold higher concentrations in sepsis patients than in controls with sterile systemic inflammation. The peptide has been found to highly activate PMN and monocytes, to induce pro-inflammatory cytokine release, to stimulate migration and to reduce cell viability. Furthermore, CAAP48-treatment leads to similar pathological changes, which are described for sepsis-induced cholestasis, e.g. down-regulation of the hepatic transport protein MRP2 and a diminished expression of endothelial VE-cadherin and F-actin, which results in a loss of barrier function.
Conclusions: Based on our previous results we propose that CAAP48 is a promising sepsis biomarker that has an active role in the pathophysiology of sepsis. However, the importance of CAAP48 as sepsis biomarker has to be further evaluated in prospectively collected patient cohorts with defined bacterial infections, different foci of infection and in the time course of sepsis to clarify and validate the role of CAAP48 in early identification of patients at risk and timely initiation of appropriate antibiotic therapy. Furthermore, studies in progress will evaluate the underlying molecular mechanisms of CAAP48 uptake, receptor binding and signal transduction.

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Infection 2017

Comparison of three different animal models for systemic inflammation

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Introduction: Different rodent models have been developed to investigate new drug candidates and to mimic systemic inflammation in humans.
Objectives: Since these models are still discussed controversially, we aimed to comparatively evaluate the most widely used models with respect to the systemic effects, the influence on organ functions and to the underlying pathophysiological processes.
Methods: Systemic inflammation was induced in C57BL/6N mice with lipopolysaccharide (LPS) treatment, peritoneal contamination and infection (PCI), or cecal ligation and puncture (CLP). Since we were interested in the natural course of the disease and since we induced a mid-grade systemic inflammation making acute lethality very unlikely, in none of the three animal sepsis models antibiotic therapy or fluid resuscitation was given. 0, 2, 4, 6, 12, 24, 48 and 72 h after inflammation onset, mice were sacrificed and blood was obtained for TNF alpha, IL-6, IL-10, IFN gamma, CXCL12, ALAT and blood glucose evaluation. Oxidative stress in the brains, kidneys, livers, lungs and spleens was assessed and liver biotransformation capacity was determined. Finally, we utilized immunohistochemistry in liver and spleen tissue to determine cluster of differentiation 3 (CD3), CD8, CD68, CXCL12, CXCR4, cleaved caspase-3 and TNF alpha expression patterns, and to assess the presence of various markers for oxidative stress.
Results: Treating mice with LPS and PCI caused a very similar course of the inflammation; however, LPS treatment elicited a stronger response. We observed a rapid increase of pro-inflammatory cytokine levels, an early onset of oxidative stress in the organs, an early decrease in blood glucose values and in biotransformation capacity, a pronounced immigration of inflammatory cell populations from the blood in the liver and spleen and apoptosis in the spleen

tissue. Mice exposed to either LPS or PCI recovered after 72 h. In contrast, CLP treatment induced comparatively fewer effects, but a more protracted course of inflammation.
Conclusions: All in all, the LPS model of systemic inflammation revealed to be most suitable when being interested in the impact of new therapies for acute inflammation, as the effects are clearly visible and the model exerts several methodical advantages. When inducing inflammation by CLP to mimic human sepsis more closely, the time period has to be chosen long enough as the treatment induces a delayed course of the inflammation. Finally, the parameters to be investigated have to be chosen very carefully in dependence of the aim of the study.

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Infection 2017

Modeling the metabolic reprogramming of macrophage activation

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Introduction: Bacterial infections can get systemic leading to sepsis, i.e. organ failure caused by an unbound immune response, and often death. Resting macrophages can polarize mainly into two distinct phenotypes: M1 (classical activation e.g. by microbial products or IFN-g) or M2 macrophages (alternative activation e.g. by IL-4). M1 macrophages can produce pro-inflammatory mediators such as TNF-a, IL-1 and nitric oxide [3] and can mediate an inflammatory response leading to a cytokine storm triggering sepsis. In contrast, M2 macrophages show a rather immunosuppressive behavior during sepsis [5].
Objectives: Hence, a central aim of critical care is to reduce M1 and induce M2 activation during the acute phase of sepsis. A metabolic switch of M1 to M2 macrophages had been described by upregulation of aconitate decarboxylase in M1 macrophages, which synthesizes itaconate from cis-aconitate [1], M1 macrophages modulate the citrate cycle such that nitric oxide is produced in the urea cycle [1], and other studies showed that a transition of M1 to M2 macrophages can be induced by IL-4 [4,6], which, takes days [4] making it unattractive for therapy of acute sepsis.
Methods: To understand the switch in regulation of metabolism, we investigate gene regulation using our previously published tool [7]. Simultaneously, we predict metabolic fluxes with our newly developed method based on mixed-integer-linear programming integrating gene expression data into flux balance models. Moreover, it contains a method to remove thermodynamically infeasible loops.
Results: Comparing the predicted metabolic fluxes in M1 to M2 macrophages, we have a model that reflects M1 macrophages upregulation of energy production by converting glucose to lactate (Warburg effect) while M2 macrophages increase flux for nucleotide biosynthesis.
Conclusions: Our study reveals promising results elucidating the metabolic and transcriptional reprogramming of M1 and M2 macrophages after bacterial activation paving the way for identifying therapeutic targets to reprogram M1 macrophages into a latent state.
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Infection 2017

Predicting Nutritional Uptakes of Bacillus subtilis By Integrating Gene Expression Profiles Into Metabolic Constrained-Based Models

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Introduction: Finding drug targets for antimicrobial treatment is a central focus in biomedical research. To discover new drug targets, we are interested in finding out which nutritional needs are important for pathogenic microorganisms in the host or under specific circumstances. Besides this, metabolic fluxes have been successfully constructed and predictions used by employing flux balance analysis (FBA) using constrained based modeling (Orth et al., Nat Biotechnol, 2010, Sharma et al., Semin Cancer Biol, 2013, Lewis et al., Nat Biotechnol, 2010).
Objectives: We develop FBA models using the stoichiometric knowledge of the metabolic reactions of a cell and combine this with experimental data, particularly gene expression profiles to identify drug targets being essential for nutritional uptake in the metabolic network of pathogenic microorganisms.
Methods: We implemented our method by using data from *B. subtilis* as a case study. We used a metabolic model (Oh et al., J Biol Chem, 2007), gene expression data (Nicolas et al., Science, 2012) and 13C experimental data (Chubukov et al., Mol Syst Biol, 2013). The data comprises of 13C labelling experiments based flux data and gene expression data for *B. subtilis* grown on 8 different carbon sources.

We developed a method based on mixed-integer linear programming (MIP) and trained the model with gene expression data. A further new method was added to reduce thermodynamically infeasible loops (TIL) to improve prediction results. Validation was performed by comparing predicted flux values with the fluxes from the 13C labelling experiments.

Results: We started feeding the model with gene expression data of each of the 8 carbon sources at a time and predicted the fluxes. By employing our method, our trained model could identify the correct major carbon sources based on gene expression profiles. We found improved flux predictions when compared to models not employing our new method of deleting TILs.

Conclusions: Our method is promising and can well predict flux predictions in the metabolic network of *B. subtilis*. This can be adapted to pathogenic microorganisms, like e.g. *S. aureus* to study metabolic networks and find interesting candidates as drug targets to be validated for in vitro experiments.

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Infection 2017

Peritoneal sepsis associated endoplasmic reticulum stress signaling and apoptosis in murine and human skeletal muscle tissue

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Introduction: Skeletal muscle (SM) wasting occurs early in the course of critical illness and impairs prognosis in affected patients [1; 2]. Key metabolic signaling pathways like insulin signaling have been shown to be involved in pathogenesis of this phenomenon [2].

The endoplasmic reticulum (ER)-stress-signaling pathway is an important regulator of metabolism and inflammation. Activation of this pathway can both be detrimental or adaptive by promoting metabolic dysregulation and apoptosis or cellular homeostasis, respectively [3]. However, ER stress signaling in SM has not been studied in critically ill patients and particularly in sepsis.

Objectives: To describe ER-stress-signaling and activation of apoptosis in SM of mice and patients suffering from peritoneal sepsis (PS).

Methods: We analyzed SM from 20 patients undergoing therapeutic laparotomy, among them ten patients suffering from PS and ten control patients with absence of high-grade systemic inflammation. In a corresponding peritoneal contamination and infection (PCI) mouse model, we isolated samples of gastrocnemius muscle from PS and control mice. The mRNA expression of ATF4, ATF6, CD68, XBP1u, XBP1s, CASP12, CHOP and BAX [4] was measured using qPCR in both cohorts.

In a subset of patients, presence of CD68+ cells and nuclear XBP1 translocation were assessed by immunohistochemistry (IHC) and apoptotic cells were stained using TUNEL assay.

Results: We found increased SM inflammation as assessed by significantly increased CD68+ macrophage infiltration and significantly increased CD68 mRNA expression in SM from both patients ($p < 0.05$) and mice ($p < 0.05$) suffering from PS compared to control individuals. In both human and murine SM, XBP1u and XBP1s mRNA as components of the IRE-1 branch of ER-stress were significantly upregulated in PS ($p < 0.05$). There were no significant differences in ATF4 and ATF6 mRNA expression in human SM ($p > 0.05$), indicating that the PERK and ATF6 branch of the UPR were not involved in early onset PS. Though we found increased mRNA expression of XBP1u and XBP1s, we were not able to detect increased protein or nuclear translocation of XBP in IHC. Enhanced SM mRNA expression of the apoptosis regulator BAX was found both in human and mouse PS compared to controls ($p < 0.05$). While CHOP and CASP12 as specific mediators of ER-stress initiated apoptosis were not upregulated, TUNEL staining revealed significantly increased apoptosis in human SM (8/8 positive in PS vs 2/6 positive in non-PS controls; χ^2-test, $p < 0.05$).

Conclusions: Sepsis leads to increased inflammation and pro-apoptotic signaling in SM. This is accompanied by transcriptional up regulation of IRE-1 branch of ER-stress pathway. Whether ER-stress promotes tissue damage or is protective should be focus of further investigations regarding critical illness myopathy.

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Hemoperfusion for rapid reduction of bacteria load: a non-pharmaceutical approach for treating drug-resistant bacteremia

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Introduction: Multidrug-resistant (MDR) pathogens threaten global health, and increase interest in-non-pharmaceutical therapies. (1) The WHO has identified 12 bacteria that pose the greatest threat, (2) including MDR Acinetobacter, Pseudomonas, and Enterobacteriaceae. Rapid administration of effective antimicrobial therapy is essential for improved outcomes (3) which correlate with reduction in both bacterial load and duration of bacteremia (4).

Objectives: In vitro and large-animal testing of 'Seraph', a broad-spectrum, biomimetic hemoperfusion device, have shown that it can quickly remove drug-resistant bacteria from whole blood. Seraph 100 is a filter containing high-capacity 'adsorbent media' that mimics heparan sulfate binding sites on cell surfaces. Oligomeric heparin is permanently attached to the beads with a covalent bond creating a 'brush-type' molecular architecture that allows the entire heparin chain to be accessible to molecular and cellular adsorbates. Seraph 200 also contains a 'supplemental adsorbent' that removes endotoxins.

Methods: In vitro testing: 2.5 mL filter syringes were filled with sterilized heparin-functional media. Two mL of pathogen-containing blood at ca. 105 CFU/mL were passed through the syringes and pre- and post-concentrations were enumerated. Microbiology testing was performed at Microchem Laboratories, Texas, USA. In vivo testing:

Drug-resistant pathogen challenges of Seraph 200 by Battelle used a swine model. Bacteria were infused continuously at concentrations between 400 and 3100 CFU/mL into a prototype extracorporeal hemoperfusion circuit. Blood samples were collected contemporaneously before and after Seraph to quantify bacteria removal by the device.

Results: In vitro results demonstrated efficient single-pass removal of drug-resistant bacteria strains: MRSA (92%), MRSE (66%), CRE *E. coli* (99.9%), CRE *K. pneumoniae* (99.9%), VRE *E. faecalis* (91%), and ESBL *K. pneumoniae* (39%). The Battelle porcine model demonstrated that Seraph 200 could continuously remove MRSA (78–88%), MDR (79%), MDR *P. aeruginosa* (83%), and MDR *K. pneumoniae* (35%).

Conclusions: In vivo pre-clinical feasibility studies have been confirmed in an ongoing German clinical trial using Seraph in a 4-h therapy during hemodialysis. Human and pre-clinical animal testing support the use of Seraph therapy as an adjunctive tool to improve the therapeutic efficiency of currently available drugs, or when no effective antimicrobial drugs are available.

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Infection 2017

Survival and function in rats with genetic predisposition for high or low exercise capacity

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Introduction: Sepsis may lead to multiple organ failure and death. Sepsis-induced cardiac dysfunction represents one of several possible complications and is co-responsible for the high mortality typical for sepsis. It is accepted that physical fitness as well as high intrinsic exercise capacity positively influences cardiovascular health. However, less is known about the impact of these factors on cardiac performance and metabolism in sepsis.

Objectives: We aimed to identify the effect of sepsis on cardiac function, metabolism and insulin responsiveness in rats differing in their genetic predisposition for either high or low inborn exercise capacity.

Methods: Sepsis was induced in 15-week old rats with high (HCR) or low (LCR) intrinsic running capacity by intraperitoneal injection of a human fecal suspension. The Clinical Severity Score (CSS) was determined to assess sepsis severity 6 and 24 h later. At 1 and 5 weeks, hearts from sepsis survivors were excised and prepared as isolated working hearts. Cardiac function, substrate oxidation and response to insulin were measured using radioactive tracer technology.

Results: The two groups did not differ in their CSS scores at 6 and 24 h and survival was poor with 33% (HCR) and 38% (LCR) survivors at 72 h. After 5 weeks, septic animals displayed substantial reductions of cardiac power (Control vs 5w of sepsis: 45.6 ± 2.9 vs. 37.6 ± 1.5, $p < 0.05$ mW/g dry). This reduction in power was not associated with sepsis-induced alterations in glucose oxidation (0.33 ± 0.06 vs. 0.30 ± 0.05 μmol/min/g dry, n.s.) or fatty acid oxidation (0.67 ± 0.08 vs. 0.70 ± 0.05 μmol/min/g dry, n.s.). However, when relating substrate oxidation to cardiac power as a measure of substrate efficacy, there was an increase in substrate used per force. Insulin response was increased in HCR only (change of glucose oxidation: $+0.32 \pm 0.08$ vs. $+0.39 \pm 0.07$ and $+0.16 \pm 0.04$ vs. $+0.51 \pm 0.11$ μmol/min/g dry, $p < 0.05$).

Conclusions: The detrimental effects of sepsis on survival in rats are not affected by their genetically determined exercise capacity. Sepsis causes significant mortality and contractile dysfunction in survivors. This dysfunction was accompanied by maintained glucose and fatty acid oxidation suggesting significantly decreased efficiency of substrate use.

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Microbiota of the albino rats ileum lumen under abdominal sepsis

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Introduction: Microflora of the distal part of ileum plays a separate role in formation of abdominal sepsis (AS) and is characterized by enhanced vascularization and localization of mucous-associated lymphoid clusters, which provides a significant ability to resorption of antigens in comparison with other parts of the gastrointestinal tract.

Objectives: To study the qualitative and quantitative composition of the microbiota of the ileum lumen of albino rats with AS.

Methods: A bacteriological method was used in 25 white rats (200–220 g). All animals were quarantined for 10–14 days. Ten rats with induced AS concluded the main group, 15 intact animals formed the control. Before the study all animals were examined for possible pathology. In sterile conditions, the abdominal cavity was opened, a portion (1.5–2 cm) of the distal part of ileum with its contents was taken. Pure cultures were identified by morphological, tinctorial, cultural and biochemical properties.

Results: In animals with experimental AS the dominant microflora of ileum lumen consists of obligate anaerobic bacteria of the genera Bacteroides, Bifidobacterium, Lactobacillus, Peptostreptococcus, facultative anaerobic and aerobic opportunistic enterobacteria: *E. coli* and Proteus; additional microflora is formed by the bacteria of the genus Enterococcus and accidental—by Peptococcus and Klebsiella. Also, it should be noticed the elimination of the genera Bifidobacterium and Lactobacillus from the iliac lumen in 20.0% of the animals and the contamination and colonization of the biotope with the conditionally pathogenic enterobacteria of the genera Klebsiella and Proteus. The results of the bacteriological study confirm the moderate deficiency of the most important for composition of the intestinal microbiocenosis and multifunctional role in maintaining the microecological homeostasis bacteria of the genus Bifidobacterium (reduction by 33.91%) and Lactobacillus (reduction by 27.53%), as well as a less significant decrease in the population level of bacteria of genera Bacteroides (19.85%), Peptostreptococcus (13.88%), Escherichia (8.97%), Enterococcus (8.97%) etc. The number of Proteus in the microbiocenosis is increased by 10.33%; Klebsiella reached a moderate population level. Changes of the population level of bacteria of different taxons lead to an imbalance of microbiocenosis of

the ileum lumen, which is more clearly indicated by other analytical indices. Thus, in Bifidobacterium the quantitative dominance is reduced by 44.88%, participation in the formation of microbiocenosis of the biotope by 50.98%; in Lactobacilli by 37.02 and 36.01% respectively, in Enterococci—by 3.5 times and 4.51 times respectively. The quantitative dominance and participation in the formation of the microbiocenosis of opportunistic enterobacteria increased by 32.37 and 57.96%.
Conclusions: AS leads to deficiency and in some cases (20.0%) to elimination of Bifidobacterium, Lactobacillus and contamination and colonization of the ileum lumen with opportunistic enterobacteria (Klebsiella, Proteus).

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Infection 2017

Role of AMPK in systemic inflammation

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Introduction: The systemic inflammatory response syndrome (SIRS) is characterised by endothelial and microvascular dysfunction resulting in decreased organ perfusion and subsequent development of organ failure. The energy-sensing enzyme AMP-activated kinase (AMPK), a crucial regulator of cell metabolism and homeostasis, is thought to play an important role in inflammatory processes. It exerts significant anti-inflammatory and antioxidant effects in a variety of cell types, in part by inhibiting the pro-inflammatory NFκB pathway.
Objectives: We hypothesised that AMPK may control SIRS and limit inflammatory responses of the endothelium thereby protecting against vascular dysfunction.
Methods: Experiments were performed in wild type (WT) mice and in mice, in which the catalytic subunit AMPKα1 was knocked out (KO). SIRS was induced by intraperitoneal injection of LPS (10 μg/g body weight). To investigate differences in WT and KO mice, plasma cytokine levels and markers of cellular damage as well as cytokine levels in the liver were analysed. To characterise the influence of AMPK on endothelial permeability in vivo, a vascular leakage assay employing Evans Blue was performed. In vitro experiments were performed in human umbilical vein endothelial cells, which were stimulated with cytokines (IL-1β, TNF-α) and/or LPS and analysed for permeability (ECIS) and adhesion molecule expression (flow cytometry). AICAR and A769662 were used to activate AMPK in these experiments.
Results: Female WT mice showed a higher survival compared to male WT mice in response to LPS. Knockout of AMPKα1 reduced survival in female mice but had no effect on mortality in male mice. In line with this, higher plasma cytokine levels (IL1β, TNFα) and increased plasma markers of cellular damage (lactate dehydrogenase) were observed in female AMPKα1 KO mice indicating protection from systemic inflammation by AMPKα1. 6 h after LPS injection, a significant vascular leakage as monitored with Evans Blue was seen in organs including liver. Here, no gender difference was observed. LPS-induced vascular leakage in liver was clearly higher in AMPKα1 KO mice compared to WT mice. In parallel, cytokine levels (IL-1β, TNF-α) in liver and plasma markers of hepatocellular damage (alanine aminotransferase, aspartate aminotransferase) were increased in AMPKα1 KO animals. These data indicate that AMPKα1 protects from vascular leakage and liver inflammation during SIRS. This may be in part due to endothelial barrier stabilisation and prevention of leukocyte immigration into the tissue. Accordingly, pharmacological activation of AMPK in cultured endothelial cells led to a significant decrease of cytokine-induced endothelial permeability and adhesion molecule expression.
Conclusions: Taken together, these data underline the importance of AMPK as an anti-inflammatory molecule in the context of systemic and local inflammation. AMPK may represent a pharmacological target to prevent or ameliorate inflammatory responses during SIRS.

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Infection 2017

TLR-expressing cells as biosensor for bacterial ligands

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Introduction: The early recognition of potentially pathogenic microorganisms is the essential step before mechanisms of innate and adaptive immune responses are initiated. The evolutionary battle between host and pathogens lead to the development of the Toll-like receptor system for the recognition of bacterial, fungal and viral components of very different chemical composition. Bacterial and fungal cell wall components, proteins, toxins and nucleic acids belong to those molecules and the binding of the so-called pathogen-associated molecular patterns (PAMPs) to Toll-like receptors (TLRs) provokes a signaling cascade resulting in recruitment of the transcription factor NF-κB. NF-κB binds to certain NF-κB response elements in the promotor region of genes involved in inflammation and immunity and activates their transcription. The recognition process takes place soon after microorganisms overcome the barriers of skin and mucosa by tissue resident immune cells carrying TLRs and also other immune receptors.
Objectives: We generated a TLR expression based cellular assay to detect bacterial ligands in liquid samples. Our intention is to use reporter cell lines to measure the activation and signalling of certain Toll-like receptors to evaluate blood plasma samples from septic patients.
Methods: We generated stable isogenic cell lines expressing single TLRs (e.g. TLR5) or combinations of TLRs and cofactors (e.g. TLR4). The cells are seeded in 96 well plates and stimulated after 14–16 h of adherence. The cells were stimulated with soluble ligands, heat-inactivated bacteria and more complex samples e.g. spiked plasma samples and blood plasma from septic patients.
Results: Cells expressing TLR2, TLR2/TLR1, TLR2/TLR6, TLR4-CD14-MD2 or TLR5 were dose-dependently activated by the according ligands lipoteichoic acid, Pam3Csk4, Fsl-1, LPS and Flagellin as well as by heat-inactivated bacteria and spiked human plasma samples. Septic plasma samples activated several TLR-expressing cell lines according to the causing microorganism.

Conclusions: An optimized version of the system may potentially be used as a biosensor system for bacterial components in the context of diagnostic procedures.

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Staphylococcus aureus pathogenesis: The role of different host cell types during the passage from sepsis to chronic osteomyelitis

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Introduction: *Staphylococcus aureus* is an opportunistic pathogen and a frequent cause of infection. A dreaded complication of sepsis is metastatic infection of different organs, such as osteomyelitis, which is longsome and difficult to treat and often requires surgical interventions. The ability of *S. aureus* to establish chronic infections most likely originates in the complex interaction of the bacteria with bone tissue that is not fully understood. It includes invasion of *S. aureus* in professional and non-professional phagocytes, expression of cytotoxic and proinflammatory factors and bacterial persistence within the intracellular location for long time periods.
Objectives: We established a mouse and human biochip organoid to analyse the bacterial localization and the bacterial-host cell communication that might trigger bacterial adaptation processes in bone tissue. We focused on non-phagocytic bone cells like osteoblasts and osteocytes
Methods: Mouse bone biochip was established by seeding MC3T3-E1 osteoblast and MLO-Y4 osteocyte murine cell lines with different coatings and orientations. Those last ones were treated under mineralising conditions to induce the development of matrix. The human bone biochip was developed using SaOS2 osteoblastic cell line, as a human osteocytes cell line is not available; we used an already published method where SaOS2 human osteosarcoma cells can differentiate into osteocyte-like cells. The differentiation cell process was analysed by fluorescence microscopy during 21 days. Cells were fixed in PFA 4% and stained with Phaollidin-AF633 and DAPI
Results: Mice and human bone biochips were established. Providing the appropriate mineralising conditions MLO-Y4 and SaOS2 cells synthesised a mineralised matrix and acquired a characteristic osteocytes dendritic morphology over the time, which was successfully measured by AlizarinRed. After 7 days under differentiating conditions, the presence of calcium was observed indicating the beginning of the mineralising process. Highly mineralised cultures were observed after 21 days. Osteoblasts and osteocytes were able to be seeded in both orientations. Both biochips presented differentiated cells and mineralised matrix as it was observed
Conclusions: The knowledge about the localization of *S. aureus* during infection is very important in order to design novel regimes to prevent and treat chronic infections. This is difficult to be analysed and all tries in animal models have failed up to now due to the fact that systemic and localized infections cannot be determined via visual observation. The biochip-organoid bone is an alternative model to study the localisation of *S. aureus* during infection by closely simulating the in vivo disease. This alternative ex vivo model will provide critical real-time information needed for developing novel therapeutics and investigate possible effective antimicrobial treatments for chronic infections

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Chemerin as an effector of transactivation of hepatic stellate cells

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Introduction: Hepatic stellate cells (HSC) have been identified as key mediators of hepatic fibrosis, a pathophysiological response of the tissue towards acute or chronic stress. Once activated by cytokines, viral infection, ethanol or other toxic agents, HSC lose vitamin A, synthesize extracellular matrix components and thus transform into contractile myofibroblasts. Adipokines such as leptin or adiponectin are also known mediators of hepatic fibrosis. Chemerin is a novel adipokine acting mainly via its own G-coupled receptor CMKLR1, playing a role in type 2 diabetes, psoriasis and rheumatoid arthritis. Interestingly, serum concentration was found elevated in patients affected by peritoneal sepsis [1]. We therefore investigated whether chemerin has an impact on the HSC-mediated liver fibrosis in an in-vitro model.
Objectives: to investigate the impact of chemerin on transactivation of immortalized human LX-2 cells monitoring intracellular signalling events and functional activity (migration, contractility and loss of vitamin A)
Methods: Experiments were performed on LX-2 cells following time and concentration dependent stimulation: (1) qPCR analyses of established marker genes of hepatic fibrosis, (2) migration assay using ThinCert™ inserts, (3) contractility assay was carried out by embedding LX-2 cells into a collagen matrix and measuring the time-dependent collagen gel size change (4) mobilisation of Vitamin A storage via Raman spectroscopy [2] (5) presence of CMKLR1 on LX-2 cells by immunoblotting, also following of sub-cellular fractionation.
Results: As a result of the pilot study, both CMKLR1 mRNA as well as the protein content were detected in (stimulated) LX-2 cells at high levels via qRT-PCR and immunoblotting. Surprisingly, chemerin failed to induce any change in the synthesis of molecular markers of activation of LX-2 cells as a surrogate of cellular transactivation. While chemerin had also no impact on the contractility, migration was inhibited in a dose dependent manner. Furthermore, chemerin induced loss of vitamin-A was observed in LX-2 cells as a cell specific marker of cellular stress. However, subsequent sub-cellular fractionation revealed lack of CMKLR1 protein in the membrane fraction.
Conclusions: Though CMKLR1 is not present on the surface of LX-2 cells, chemerin effectively and dose-dependently inhibited the migration of LX-2 cells and lead to the loss of vitamin A. As selective receptor signalling properties have recently been described for GPR1 [3], one can assume that the chemerin-induced activation of LX-2 cells is based on alternative, CMKLR1-independent pathways. Since both migration as well as loss of vitamin A are hallmarks of transactivation of LX-2 cells, we suggest that chemerin plays a role in the development of hepatic fibrosis. However, further experiments with primary hepatic stellate cells or animal models are needed. Furthermore, the results of this study reveal the need of extended research regarding the molecular mechanisms of chemerin.

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Infection 2017

An immunoproteomic approach to identify protein candidates for pathogen detection and risk stratification in sepsis patients

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Introduction: Sepsis, "as life-threatening organ dysfunction caused by a dysregulated host response to infection" (Singer 2016), is the third leading cause of death in hospitalized patients in Germany. Due to an alarming increase of antibiotic resistant bacteria rapid microbial diagnosis is needed to ensure the most appropriate antibiotic is chosen. Blood culture, the gold standard of pathogen detection, is positive in less than half of the cases of clinical sepsis. In contrast, PCR-based methods promise to be faster, but carry a risk of false positive results.
Objectives: To complement conventional microbiological diagnosis, we plan to develop a multiplex immunoassay. This assay will be based on the quantification of the antibody binding kinetics to several bacterial proteins simultaneously.
Methods: In a prospective clinical trial (IMI_Sep, BB020/13) we collected plasma samples of sepsis patients before the onset of sepsis, at diagnosis and during the infection. The most frequent sepsis pathogens isolated from blood cultures or microbiological samples of the septic focus were *S. aureus, S. epidermidis, E. coli, E. faecium, P. aeruginosa, S. marcescens* and *K. pneumoniae.* The bacteria were cultivated until stationary growth phase and extracellular proteins were precipitated from the supernatants. Patient plasma antibody binding to the extracellular proteins of the corresponding infectious agent were quantified by using a Simple Western Assay (ProteinSimple®). Patients with an increase of antibody binding during infection by at least a factor of two were defined as patients with an informative course of disease. Extracellular proteins of sepsis pathogens from patients with an informative course of disease were separated by 2D gel electrophoresis, transferred to a PVDF membrane and incubated with plasma. Total antibody binding was detected by using chemiluminescence.
Results: For each patient, two 2D immunoblots were performed to compare antibodies in plasma taken before sepsis onset with those in plasma obtained at a later time point. During the disease course, often new spots appeared on the 2D immunoblots and intensities of other spots increased. For each of the six bacterial species at least five immunogenic proteins were identified. These proteins will now be cloned, recombinantly expressed and included in a multiplex assay.
Conclusions: This multiplex immunoassay may complement the microbiological and sequence-based diagnosis of sepsis-pathogens with information about the specific immune response. This promises new insights into the role of the adaptive immune response in infection control. The assay may also be suitable for stratification of patients according to their risk of infection or complications of infection, such as sepsis. Finally, the assay might support the diagnosis of chronic implant-associated infections.
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Characterisation of two different preclinical murine models of haemolytic uraemic syndrome

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Introduction: Diarrhea-positive haemolytic uraemic syndrome (HUS) is a rare kidney disorder most frequently caused by infections with enterohaemorrhagic *Escherichia coli* (EHEC). Clinically, it is characterised by a triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury (AKI). Shiga toxins (Stxs), particularly Stx2, as key virulence factors of EHEC play a predominant role in HUS development. Studying renal endothelial damage in infection-associated HUS will allow us to gain fundamental insights into pathophysiological principles of disturbed microcirculation and thrombosis—a hallmark of HUS and septic shock.
Objectives: Specific prognosis-improving treatable targets in EHEC-associated HUS are currently not available. In this study, we aimed to establish and characterise an appropriate murine in vivo model to elucidate molecular mechanisms underlying HUS pathogenesis and perform pharmacologic intervention studies.
Methods: 10-16 week-old male C57BL/6J wild-type mice obtained either a single high dose of Stx2 (acute model) or three low doses on days 0, 3 and 6 (subacute model) accompanied by volume resuscitation. Disease progression was monitored. Kidney dysfunction and damage were characterised by analysis of plasma urea and creatinine, histological evaluation and detection of specific immunohistochemical targets for kidney and endothelial damage, apoptosis, proliferation, complement activation and immune cell invasion. Differences in gene expression patterns were examined using microarrays.

Results: Plasma creatinine and urea were significantly increased in Stx2-challenged mice in both models, indicating kidney dysfunction. Histological and immunohistochemical investigation of renal sections revealed significant kidney damage accompanied by loss of endothelial cells and thrombotic microangiopathy as hallmarks of human HUS in both the acute and subacute model. In contrast, complement activation, immune cell invasion and higher levels of apoptosis as well as proliferation were observed only in the subacute HUS model. Microarray analysis of both models showed 91 overlapping genes regulated by Stx2-challenge.
Conclusions: We established and characterised two distinct preclinical models of HUS. Our results indicate that the subacute model better reflects human HUS by presentation of kidney dysfunction, thrombotic microangiopathy and endothelial damage but also complement activation accompanied by immune cell invasion. The models allow further pathophysiological characterisation and subsequent pharmacological studies in HUS and related conditions associated with impaired microcirculation in the kidney.
W.P. and S.D. contributed equally

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Influence of Mitochondrial Function on Sepsis Severity and Outcome

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Introduction: Sepsis is associated with cardiomyopathy and mitochondrial dysfunction, which may predict sepsis mortality. However, a temporal relation between cardiac and mitochondrial function has not been investigated yet.
Objectives: The objective of this study was to evaluate the influence of sepsis on cardiac and mitochondrial function in a time dependent manner.
Methods: Sepsis was induced by intraperitoneal injection of human feces (peritoneal contamination and infection model - PCI) in wild type and transgenic mice. After 6, 24 and 72 h, sepsis severity and cardiac function were assessed. Furthermore, mitochondrial function of heart, skeletal muscle and liver was investigated at these three time points. Mice without PCI served as a control.
Results: In wild types, PCI resulted in severe sepsis within the first 24h with elevated clinical severity score (basal vs. 24 h: 4 ± 0 vs. 11.5 ± 1.1), reduction of body weight and mortality rates of 0% after 6h, 62% after 24 h and 90% after 72h. Six hours after the septic insult diastolic dysfunction (E/DT basal vs. 6h: 30.3 ± 1.8 vs. 19.5 ± 1.7; $p \leq 0.5$), cardiac output reduction (17.3 ± 1.0 vs. 11.9 ± 0.8; $p \leq 0.5$) and heart rate elevation (453 ± 11 vs. 516 ± 14; $p \leq 0.5$) was measured, with no impairment of ejection fraction (53.8 ± 1.8 vs. 56.6 ± 1.9). Changes in cardiac function recovered slightly after 24 h and fully after 72 h in the survivors. At 6 h, there was no difference in any echocardiographic parameter between the 24 h-survivors and the non-survivors, making a selection bias unlikely. In contrast, cardiac state 3 respiration remained unchanged until 6 h but was significantly decreased after 24h and even more so after 72 h (basal-6 h-24 h-72 h:304 ± 44 vs. 276 ± 58 vs. 224 ± 36 vs. 116 ± 28 natomsO/min/mg). Therefore, we analyzed mice with a moderate overexpression of SirT1, an activator of mitochondrial biogenesis. Cardiac state 3 respiration was higher at baseline in transgenic mice and was not affected by sepsis (basal-6 h-24 h-72 h: 457 ± 60 vs. 545 ± 46 vs. 460 ± 27 vs. 530 ± 59natomsO/min/mg). Furthermore, cardiac function was less impaired with sepsis (cardiac output: basal vs. 6h: 19.1 ± 1.0 vs. 15.3 ± 0.8; $p \leq 0.5$). However, despite protected mitochondrial function, sepsis mortality rate and sepsis severity were not improved.
Conclusions: Our data indicate, that there is no causal relation between mitochondrial function and sepsis severity.

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QUANTIM—quantification of the innate immune function in whole-blood infection assays

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Introduction: Despite the availability of the wide range of data on cellular and molecular processes involved in systemic inflammation, little progress has been made in pharmacologically modulating the inflammatory dysregulation that occurs in sepsis. A major reason for this is the heterogeneity of sepsis as a clinical syndrome, resulting from highly diverse pathological conditions and showing variable disease kinetics in individual patients. Therefore, it is of importance to develop tools that enable categorization of septic patients and predict the efficacies of tailor-made therapeutic interventions.
Objectives: Within this project, we use a human whole-blood assay of infection combined with advanced mathematical modeling to answer the two following questions:
Are there pathogen-specific patterns of immune activation during whole blood infection?
Are there immune effector functions that allow stratification of sepsis patients?
Methods: Blood samples taken from healthy donors and patients were inoculated with the two model pathogens *S. aureus* and *C. albicans.* After a 4 h time course immune cell activation and the association of the pathogens with different immune cells were determined using differential FACS staining. Based on time course data on pathogen distribution to different immune cells a virtual infection model was used to perform detailed and quantitative predictions on the dynamics of host-pathogen interaction.
Results: First, the infected blood samples from healthy donors provide a comparative analysis of the regulatory networks governing inflammation and pathogen elimination. We found clear differences in the immune response, e.g. the rates of immune cell reaction differ considerably for infection with *S. aureus* and *C. albicans*. Thereafter, the whole-blood assay was performed with a more homogeneous population than septic patients. Within a pilot study, blood samples from patients who underwent cardiac surgery with extracorporeal circulation were examined. This surgery provides an inflammatory stimulus that is both time-defined and relatively homogeneous. With the ability to investigate the blood of the same patient at defined time points before and after extracorporeal circulation, inter-individual differences and the effects of inflammation can be clearly distinguished. The analysis of three patients displayed an increase in white blood cell count after surgery as well as variations in immune cell reaction rates and immune cell activation. Moreover, pathogen specific pattern could be detected for the immune responses before and after surgery.
Conclusions: Once optimized, analyses of blood samples from sepsis patients and patients who have survived severe sepsis will follow.

This will allow identifying patterns of the dysregulated immune homeostasis providing functional classifiers for the differentiation of sepsis patients, thereby forming a basis for future patient stratification.

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Caspofungin modulates ryanodine receptor-mediated calcium release in human cardiac myocytes

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Introduction: Caspofungin (CAS) is known as an effective and safe antifungal agent for the prophylaxis and treatment of different cohorts of patients (1). Recent studies revealed that CAS might alter cardiac function (2,3,4). Especially critically ill patients might be at risk for hemodynamic impairment during CAS therapy.
Objectives: Based on our previous studies of cardiac impairment after CAS application in endotoxemic rats and isolated rat cardiac myocytes, the aim of our present study was to examine the mechanisms behind CAS induced cardiac alterations.
Methods: For this purpose human cardiac myocytes (HCM, Promocell, Heidelberg, Germany) were incubated with different doses of CAS (75, 100, 130, 140, 150, 200 µg/ml) for 30 min. We performed cytosolic calcium imaging using 2.5 µM Fura-2 in calcium-containing and calcium-free media and measured the intracellular calcium concentration ([Ca2+]i) by detecting the changes in 340/380nm Fura-2 fluorescence intensity ratio as described before (5). Furthermore, we recorded the oscillation frequency (s-1) in CAS (12.5, 25, 50, 100, 200 µg/ml) treated adult rat cardiac myocytes compared to untreated controls. Intracellular [Ca2+] storages were depleted using 30 mM caffeine (CAF). Ryanodine receptors were inhibited using 40 µM ryanodine (Ry).
Results: Caspofungin treatment in HCM caused a dose dependent increase in [Ca2+]i. The CAS dependent [Ca2+]i increase was also found in experiments in calcium-free buffer medium (nCTRL = 20; nCAS = 30-50 per group; ($p < 0.01$) of [Ca2+]i with CAS >100µg/ml and increased [Ca2+]i between 2.6 and 4.5 fold (control<2 fold)). The dose-effect analysis revealed ED50 = 146.7 [µg/ml] and ED50 = 113.2 [µg/ml] within calcium-containing medium or calcium-free medium, respectively.
Regarding oscillation frequency (s-1) measurements in adult rat myocytes, we found significantly ($p < 0.01$) elevated mean values among experimental groups with CAS >25 [µg/ml] compared to untreated controls (nCTRL = 24; nCAS = 10-22 per group, 12.5 µg/ml: FC = 1.13, p = 0.05; 25 µg/ml: FC = 1.11, p = 0.02, 50 µg/ml: FC = 1.60, $p < 0.001$, 100 µg/ml: FC = 2.28, $p < 0.001$; 200 µg/ml: FC = 2.74, $p < 0.001$).
CAS [140µg/ml] induced liberation of Ca2+ was significantly reduced in the presence of CAF [nCTRL = 20, nEffect = 40; CAF+ vs. CAF-: FC = 1.04, p = 0.7] or Ry [nCTR = 20, nEffect = 40; RYN+ vs. RYN-: FC = 1.15, p = 0.06].
Conclusions: In our study, we found a dose dependent increase in [Ca2+]i after CAS treatment. Ca2+ ions were found to be released from intracellular caffeine sensitive stores most probably via activation of ryanodine receptors. Further studies are needed to explore the underlying mechanism how CAS activates ryanodine receptors.

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Differentiation of the small colony variant phenotype of *Staphylococcus aureus* from the wild type using Raman spectroscopy

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Introduction: *Staphylococcus aureus* is one of the most common Gram positive bacteria found in case of sepsis [1]. Furthermore these bacteria are able to cause relapsing infections by persisting intracellularly [2]. Characteristic for this behaviour is the phenotypical switch between wildtype and the small colony variant (SCV) phenotype. SCVs are adapted to the intracellular milieu by having a reduced metabolism, slow growth and a decreased exotoxin secretion. In this intracellular location the bacteria are well protected against the host immune system and antimicrobial treatments and might be a reservoir for chronic infections [2].
The common method to differentiate between wildtype and SCV is to isolate the bacteria from the tissue and plate them on blood agar. As the SCV's grow very slowly, this will take 48–72 h before the colonies are visible.
Objectives: In our experiments we want to introduce Raman spectroscopy as a fast, label-free and non-invasive method for differentiating wildtype and SCV phenotypes.
Methods: Raman spectroscopy measures the inelastic scattering of light. The sample is irradiated with monochromatic laser light and the so formed spectra contain fingerprint-like information. These can be analyzed using statistical methods such as principal component analysis (PCA) and linear discriminant analysis (LDA). Recently we could demonstrate that this technique allows the identification of *S. aureus* directly inside endothelial cells [3].
In our case we gathered the spectra for 18 strains of *S. aureus*. Five pairs of wildtype and the corresponding stable SCV were used as training data to build a classification model. Five strains of stable SCV's gathered from patients with chronic infections were used as independent test data. Furthermore three standard wildtype strains were used for the validation of the model.
Results: The training data have been analyzed using PCA and LDA, revealing the most significant differences in the spectra of the two phenotypes. The test data could then be differentiated with an accuracy of 97.4%.

Conclusions: The characteristic variances between wildtype and SCV could now be used as marker bands to identify the phenotype of intracellular bacteria in further experiments.
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In depth localization of *Staphylococcus aureus* in a hematogenous bone infection mouse model using two-photon microscopy

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Introduction: *Staphylococcus aureus* is one of the most frequent pathogens found in osteomyelitis with around 80% of cases. Hematogenous osteomyelitis results from bacteria spread through the bloodstream into the bone and can occur as a sequela of sepsis. The bacteria are able to adapt to several kinds of host tissues through different virulence mechanisms such as sigB (1). They also adapt to the bone and can persist there leading to chronic osteomyelitis. This disease is associated with severe, painful bone deformations and is difficult to treat with antimicrobial substances.
Objectives: We want to use advanced imaging techniques to study the pathogenesis of *S. aureus*-mediated hematogenous osteomyelitis in an established mouse model. Thereby we aim to identify the host cells and parts of the bone where the bacteria establish their niches.
Methods: Mice were infected with *S. aureus* strain 6850 as described in (1). The mice were sacrificed after 1 week (acute osteomyelitis) and after 6 weeks (chronic osteomyelitis). Isolated bones from legs were fixed in 4% paraformaldehyde and decalcified with 14% EDTA for 7 weeks. Thereafter, thick cryosections of 100 µm were generated and stained by immunofluorescence labelling of *S. aureus* using an adapted protocol of (2). Cell nuclei were counterstained with DAPI. Labelled, mounted sections were scanned by two-photon microscopy using a Ti:sapphire laser (Coherent, Germany) coupled to an LSM780 microscope (Zeiss, Germany). A 63x C-Apochromat/NA 1.15 long working distance objective (Zeiss, Germany) was used for optimal resolution.
Results: *S. aureus* could be successfully detected with two-photon-microscopy in several µm depths of the section. Compared with normal laser scanning microscopy, the signal-to-noise-ratio and contrast was much better using the two-photon excitation. We were able to detect *S. aureus* in different parts of the bone mainly located around cell nuclei which suggests intracellular state in both acute and chronic infection. Deformation of bones in chronic osteomyelitis was clearly visualized by X-ray imaging and correlated to the fluorescence images obtained from the bone sections. In those regions high numbers of bacteria in the connective tissue as well as in the hard bone were found in specific clustering areas.
Conclusions: We could demonstrate that two-photon microscopy is a superior tool for giving high contrast and resolution images of the small bacteria in deep layers in thick tissue sections. Further studies using labelling of different bone markers will provide information about the intracellular location, the dynamics of bacteria movement and adaptation from acute to chronic osteomyelitis.
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Synaptic dysfunction and long-term cognitive deficits in a mouse model of polymicrobial sepsis

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Introduction: Sepsis survivors often suffer from prolonged cognitive impairment and deficits in executive function. Due to the continuous improvement in intensive care and the resulting higher surviving rate of patients this cluster of symptoms will be of increasing economic relevance in the future. To date an effective treatment is lacking and the underlying pathomechnisms are unknown.
Objectives: Aim of the study was the analysis of cognitive impairments and correlating abnormalities in neuronal function and plasticity in a mouse model of long-term survival of sepsis. Further, we tried to elucidate abnormalities in the cellular pathways to identify potential therapeutic targets.
Methods: A modified peritoneal contamination and infection model (PCI) was used as a model for polymicrobial infection. 6–8 weeks after PCI-induction surviving animals were subjected to serveral multidimensional behavioral tasks test to analyze basic health parameters, locomotion, anxiety-related behavior, and spatial learning. Synaptic function and plasticity were analyzed in acute hippocampal brain slices by field potential recording and induction of long-term potentiation, as well as whole-cell patch-clamp recordings. Unbiased gene expression analysis and elaborated network modeling was used to identify cellular pathways
Results: We found no obvious abnormalities in surviving mice after recovery of 6 weeks by a standard phenotypic screen. Further, there were no signs of ongoing inflammation detectable. Animals of the PCI group showed significant reduced performance in the Morris water maze and in the Barnes maze in comparison to sham treated animals. Further, mice after PCI had increased level of fear-related behavior. The cognitive impairment correlated with the strength of the initial disease course after PCI. Synaptic plasticity in the Schaffer-collateral CA1 pathway was impaired and spine density was reduced in the PCI-group. Additionally, signaling cascades involved in synaptic plasticity were affected in PCI animals as revealed by western blot and unbiased gene expression study. These impairments were reversible by enriched environment.

Conclusions: Strong peripheral polymicrobial infection induces long-lasting cellular and synaptic changes in CNS neurons leading to cognitive impairments which are reversible by enriched enviroment.

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Characterization of an sphingosine 1-phosphate (S1P)-neutralizing L-aptamer (Spiegelmer) and its application in a mouse model of septic shock

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Introduction: S1P is a lipid metabolite which can activate five G protein-coupled receptors designated S1P receptors type 1–5 (S1P1-5). Dysfunction of the endothelial barrier is a major cause of cardiovascular collapse and resulting in hypoperfusion. We hypothesize that blocking S1P and/or its leakage from circulation in the acute phase of sepsis may offer a first and new treatment option in septic shock.
Objectives: NOX-S93 is characterized by its S1P binding and blocking capabilities in cell culture experiments and in the mouse model of septic shock.
Methods: Polymicrobial sepsis is induced by peritoneal contamination and infection (PCI) in mice. Changes in body temperature, hemolysis level, plasma albumin, S1P levels in blood, cytokines, and parameters of vital organ function were analyzed. Meanwhile, the effects of NOX-S93 to extract S1P from the outer RBC membrane, to prevent S1P-induced receptor internalization and to prevent S1P from dephosphorylation were investigated in cell culture experiments.
Results: 6 h after sepsis induction, NOX-S93 treated mice showed a reduced temperature drop compared to untreated PCI mice. Injection of 11.4 mg/kg NOX-S93 revealed an increase of 0.5 μM, and 57 mg/kg NOX-S93 resulted in an increase of 4 μM in plasma S1P levels compared to vehicle controls. Hemolysis and cytokines, such as interleukin (IL)-1 and IL-6 increased in PCI mice ($p < 0.05$), with no statistically significant difference between NOX-S93 treated and untreated PCI mice. PCI mice had lower albumin levels in plasma compared to control mice ($p < 0.05$), which was inhibited by treatment with 57 mg/kg NOX-S93. No statistically significant differences in parameters of organ functions, for example, blood urea nitrogen were found among control mice, NOX-S93 treated and untreated PCI mice. However, there was a tendency to higher levels of parameters of clinical chemistry of organ dysfunction in PCI mice compared to control mice and NOX-S93 treated PCI mice.
After 2 h incubation with red blood cells (RBC), we found that 10 μM NOX-S93 can extract 8 μM S1P from RBC. In hemagglutinin (HA) epitope-tagged S1P1 (S1P1-HA) rat hepatoma HTC4 cells, we found that 5 μM NOX-S93 was able to block all the S1P1 internalization caused by 0.5 μM S1P. In HTC4 wild type cells after overnight incubation with 1 μM NOX-S93 and 1 μM S1P, we observed that 1 μM NOX-S93 can protect about 400 nM S1P from degradation.
Conclusions: Based on our current data, NOX-S93 treated PCI mice had alleviated body temperature and higher plasma albumin, indicating a better endothelial barrier function, compared to untreated PCI mice. NOX-S93 was able to extract S1P from the outer RBC membrane at an equimolar ratio and can prevent S1P-induced receptor internalization and extracellular dephosphorylation of S1P.

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The protective role of sphingosine 1-phosphate in genotoxic stress induced DNA damage response in inflammation

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Introduction: The DNA damage response (DDR) is an important cellular mechanism induced by several internal and external stimuli. Depending on the extent of damage, the cell initiates cellular mechanisms, like apoptosis and autophagy, which influence the cell fate. Related to this, the sphingolipid rheostat emphasizes that sphingosine 1-phosphate (S1P) is able to protect cells from apoptosis while its related metabolite ceramide induce predominately apoptosis. The fact that low-dose genotoxic stress was shown to attenuate experimental sepsis via the induction of the DDR and autophagy in peripheral tissue cells suggests that the sphingolipid rheostat is involved in that regulation. We postulate that low-dose genotoxic stress stimulates the activation of the DDR and anti-apoptotic S1P-mediated pathways.
Objectives: Our aim is to define the role of S1P in cellular adaptions responsible for the protective effect of low-dose genotoxic stress in inflammatory responses. In particular we identified the activation of the DDR by low-dose genotoxic stress and a potential role of S1P.
Methods: Dose-dependent effects of epirubicin- induced genotoxic stress were evaluated in lung epithelial cells in cell culture experiments upon lipopolysaccharide (LPS) stimulation. Additionally, these cells were stimulated with S1P. The activation of the DDR was investigated by Western-blot and immunofluorescence microscopy. In particular we analyzed the members of the Mre11-Rad50-Nbs1 (MRN) complex as part of the double strand break response mechanism, the Ataxia-telangiectasia mutated (ATM) kinase and ataxia telangiectasia and Rad3 related (ATR) protein as central players of the DDR, as well as the DNA damage marker gamma H2AX (phospho S139). To detect the extent of DNA fragmentation, Comet assay and TUNEL assay were performed.
Results: We demonstrate that the DDR is activated after stimulation with low doses of epirubicin and S1P, and/or under inflammatory conditions. We show a dose dependent activation of the protein kinases ATM and ATR. Only low doses of epirubicin and S1P under inflammatory conditions show less activation of ATM. Furthermore we indicated that only low doses of epirubicin and S1P originate under inflammatory conditions a cytoplasmic occurrence of ATM. Concurrently the DNA damage marker gamma H2AX showed less activation to low doses and S1P stimulation, which implies less DNA damage. Similar to low doses of epirubicin, S1P stimulated cells showed also lower extent of DNA fragmentation indicating also reduced cellular damage. The members of the MRN complex are constantly expressed independent from the stimulation. Only under inflammatory conditions we identified a higher expression.
Conclusions: We demonstrate that the DDR activated by S1P shows the same protective effect like the low-dose genotoxic stress induced DDR. Furthermore the activation of the DDR and its members by S1P

stimulation indicates S1P as a potential therapeutic target to increase the cell survival under inflammatory conditions.

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Signaling dynamics and function of sphingosine 1-phosphate (S1P) receptors

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Introduction: Sphingosine 1-phosphate (S1P) is a signaling phospholipid. It is a ligand specific for five G-protein-coupled cell surface receptors designated as S1P1-5. S1P regulates pathophysiological processes involved in sepsis progression, including endothelial permeability, cytokine release and vascular tone. The maintenance of endothelial cell (EC) barrier is dependent on the S1P1 activation through S1P (Gi coupled signaling pathway) while high S1P concentrations leads to S1P1 internalization and destabilize the EC barrier. FTY720-P, a S1P agonist, also internalizes the S1P1 but it does not compromise the EC barrier.
Objectives: In this study we will define how S1P and its synthetic agonist, FTY720 differs in post translational S1P1 and S1P3 receptor regulation and signaling. On the other hand we will also investigate the receptor dimerization and clustering of S1P1 with other S1P receptors.
Methods: In order to investigate S1P1 receptor regulation in more detail, fusion proteins consisting of fluorescence proteins (mCherry/eGFP) combined with S1P1 and S1P3 are generated. These constructs will be used for overexpression predominantly in endothelial cells, particularly in the endothelial cell line EA.hy926. Protein-protein interactions (e.g. receptor dimerization and clustering) will be investigated with Förster resonance energy transfer (FRET) analysis. To study the function of transfected plasmids and potential clusters of different S1P receptors will be tested by intracellular calcium (Ca2+) flux experiments. Then the cellular trafficking and expression of S1P receptors will be determined by flow cytometry and fluorescence microscopy.
Results: Initial experiments with EA.hy926-wildtype and—HAS1PR1 transfected cells in Ca2+-measurements indicates different signaling from S1P and FTY720-P through S1P1/S1P3. S1P activates Gi via S1P1 but Gq via S1P3 which leads in a higher Ca2+-signal. In contrast FTY720 activates Gi in both receptors which results in a low Ca2+ response. FTY720 not only activates Gi via S1P3 may also stabilize EC barrier function which is normally mediated via S1P1. These preliminary barrier studies were performed by Electric Cell-substrate Impedance Sensing (ECIS). Further experiments will be done using the fusion proteins generated for FRET to analyze protein-protein-interactions e.g. receptor dimerization and clustering.
Conclusions: FTY720 not only internalize S1P1 but on the other hand it could also stabilize EC barrier via Gi S1P3 on comparing with S1P. Because of this FTY720 could be a valuable target for pharmacological interventions in the treatment of sepsis to maintain EC barrier.

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To good for the bin—data mining for secondary analysis of (pre)clinical research

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Introduction: Normally data acquisition starts based on a hypothesis. If it does not confirm, the supposed useless data end up in the bin. However, numerical algorithms without any bias may not only help finding new questions from existing data but even derive strongly advising hints for unexpected associations. Every (in)conceivable combination of data—without any limits to size—should be treated as if it were the hypothesis. This particular approach to analyze big data is called non-real-time data mining.
Objectives: The aim of the project was to create a mathematically clean, unbiased and highly automated approach—tailored for the needs of secondary analysis of (pre)clinical research, including the possibility of non-linear functional relationship which could not be found in traditional correlation. It was intended to reveal (un)known markers for systemic inflammation.
Methods: The prerequisite for each data mining is the existence of an almost homogeneous data base. In our example it was formed by overall 45 parameters (vital, blood and plasma parameters) measured in 12 individual experimental studies using 57 rats without and 63 rats with systemic inflammation following lipopolysaccharide infusion. Data were collected in an unsorted, partly incomplete and equally weighted manner. For each rat 4 classifiers (study, group, survival, time) were used to get valid samples by a later filtering of the statistical base. Any information about the hypothesis leading to the respective studies was suppressed. In order to assess whether a statistical relationship exists, a total of 6 different functional prototypes were postulated and examined for their regression. For each parameter combination, the best function type was selected. Thus, regression quality, correlation and significance were obtained in form of matrices, which can be used directly either individually or in combination for visualization or for answering complex questions.
Results: In our example, ultimately 425,250 regressions were optimized, automatically evaluated and filtered. Calculation time was about 1 h for each time point. The algorithm suggested 41 parameter combinations with the most obvious context in a first step, which were prepared for manual reading to decide whether they are nonsense, trivial, known or even new.
Conclusions: The developed algorithm is able to reveal statistical relationships from a nearly crude data base with low effort by systematic and unbiased analysis. The finding of well-known correlations proves its reliability, whose validity could be increased by clean aggregation of different studies. In addition, new interesting hints for future research could be gained. Thus, unknown markers could be found which are associated with an increased risk of death during systemic inflammation. A further development of the program is planned including multiple regressions (more than two parameters could be related to each other) or cluster analysis.
Reference: (1) Nelder JA and Mead R. A simplex method for function minimization. Comput J 1965, 7: 308. (2) Iavindrasana J, Cohen G, Depeursinge A, Müller H, Meyer R and Geissbuhler A. Clinical data mining: a review. Yearb Med Inform 2009, 121. (3) Binder H and Blettner M. Big data in medical science—a biostatistical view. Dtsch Arztebl Int 2015, 112: 137. (4) Deuel JW, Schaer

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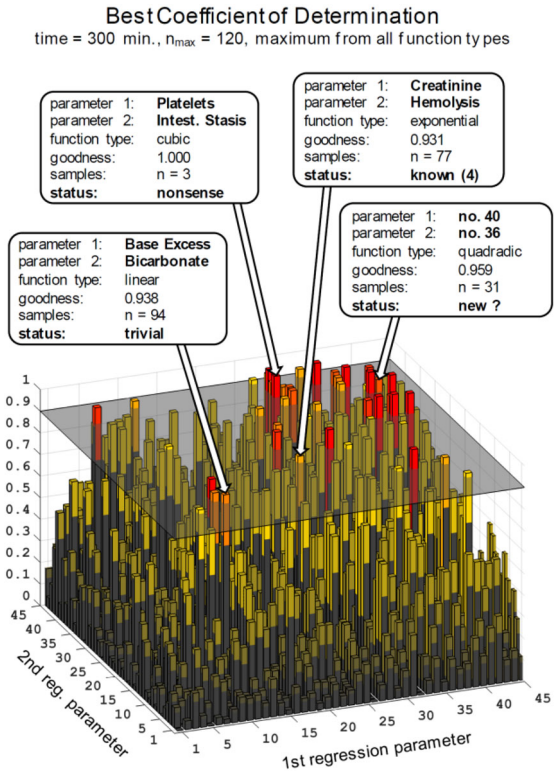
Acknowledgement: Data preparation was done in Excel, computation in C and visualization using MATLAB.



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Sphingolipid metabolism as a trigger for age-related sepsis susceptibility

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Introduction: Sphingolipids have an important role in cell metabolism and intracellular/extracellular signaling. Some components of the sphingolipid-metabolism, especially ceramides, sphingosine and sphingosine 1-phosphate (S1P), can trigger cellular differentiation, apoptosis, proliferation, cell-cycle arrest, and also inflammation. A dysregulated sphingolipid metabolism and signaling also affect sepsis, amongst other diseases. Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Sphingolipids are involved in the pathogenesis of sepsis by regulating antigen presentation, lymphocyte egress, and maintenance of vascular integrity. Clinical studies have proven that there is an age-related increase of sepsis hospitalization and an increasing incidence of severe sepsis, especially in patients over 65 years of age.
Objectives: There is a lack of knowledge about age-related differences in the sphingolipid metabolism and/or signaling and the effect of these differences on sepsis susceptibility. This study addresses the question if the sphingolipid metabolism can influence the susceptibility to sepsis, based on age-related differences in the sphingolipid metabolism of young and aged mice.
Methods: We analyzed gene expression and protein levels of enzymes involved in sphingolipid metabolism and signaling in liver samples of young and aged mice with qPCR and Western blot. Further we characterized metabolites of sphingolipids by liquid chromatography coupled to triple-quadrupole mass spectrometry (LC-MS/MS) in plasma samples.
In addition we isolated primary mouse hepatocytes from young and old wild type mice as well as from knock-out models of sphingolipid metabolizing enzymes. We performed two metabolic assays in vitro [MTT and neutral red uptake (NRU)] under lipopolysaccharide (LPS) stimulation and pharmacological manipulation of the sphingolipid metabolism and signaling.
Results: In liver samples from young and aged mice we found changes on mRNA as well as on protein levels of the two S1P-generating sphingosine kinases (SphKs). In addition we observed down-regulation of the S1P receptor type 3 (S1P3), which is one the five G-protein-coupled S1P receptors. Further we found reduced S1P levels in plasma of aged mice compared to young ones. In addition we observed in first experiments altered responses of primary hepatocytes from aged mice in comparison to young ones after stimulation with lipopolysaccharide in viability.
Conclusions: With additional analysis of primary hepatocytes from knockout-mice for specific genes of the sphingolipid metabolism or signaling, and with pharmacological manipulation of the sphingolipid pathways in this cells we will be able to draw a well-founded conclusion about the role of S1P signaling and/or metabolism in age-related phenotypes of the liver.

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Infection 2017

Complement, Immunoglobulins and Sepsis

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Introduction: The complement system plays a key role in severe diseases such as sepsis, biofilm associated inflammation, multiple sclerosis, and cancer. Increased activity of the complement factors is associated with the uncontrolled inflammation leading to tissue damages and organ failure. The most potent immunoregulatory complement factors are anaphylatoxins, which excessive release induces chemotaxis of immune cells and increases endothelial permeability affecting adversely patient's outcome. Immunoglobulins (Igs) act as major regulators of the complement. In this regard, IgM activates the classical cascade but also enhances the scavenging of membrane-bound complement factors down-regulating the complement activity.
Objectives: The evidence of therapeutic impact of Igs in complement regulation addressing its role on various diseases such as sepsis, cancer, and multiple sclerosis, and experimental evaluation of the effect of two approved Ig-products (Pentaglobin and Intratect) on bacterial of *S. aureus* and *E. coli*, both are associated with bacteremia, chronical diseases and sepsis.
Methods: An extensive analysis of publications to support the hypothesis that complement is regulated by Igs, thereby modulating the inflammatory response and outcome in different diseases has been performed. In in vitro experiments, the effect of Pentaglobin (Ig-mixture with enriched IgM) and Intratect in clearance of biofilm-

embedded bacteria by leukocytes has been investigated by determination of the viable bacteria (CFU/mL) and the immune-response after different treatments. Additionally, synergism with colistin, which seems to have an anti-biofilm activity was analyzed.
Results: The therapeutic use of Igs improved the outcome for patients with severe sepsis, transplant rejection, multiple sclerosis and cancer. In in vitro experiments, Pentaglobin reduced the biofilm-embedded *S. aureus* by 90%, whereas Intratect led to a reduction by 30–50%. Both Ig-products showed less effect on clearance of *E. coli*. The analysis of the cytokines levels is ongoing and will be presented at the conference. Colistin showed an antagonistic effect in combination with the Igs.
Conclusions: There is a strong evidence, that IgM has a beneficial effect on patient's outcome in severe disease with are associated with tissue damages due to undamped inflammation as observed in sepsis. *S. aureus* bacteremia which often results in sepsis and death might be triggered by cell debris resulting from *S. aureus* toxins. To overcome the undamped inflammation, IgM might be adminitred in addition to the anti-microbial treatment. Thereby, the antagonism with the antibiotics should be known. In case of colistin, we hypothesize that its inhibits the leukocytes. To sum up, the therapeutic efficiency of polyclonal and monoclonal antibodies needs to be further evaluated.

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Infection 2017

Integration of natural killer cells in a microfluidically perfused liver biochip

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Introduction: Natural killer (NK) cells are the main lymphocyte population in the human liver with up to 50% of the lymphocyte population. The liver acts as an innate immunity-dominant organ and NK cells therefore provide the first line of defense against pathogens and infections. NK cells highly express purinergic receptors which are activated by extracellular nucleotides (e.g. ATP, adenosine).
Objectives: The major aim of this study was to integrate NK cells into an existing microfluidically perfused liver biochip. Furthermore, isolated NK cells were investigated regarding purinergic receptors.
Methods: NK cells from human blood and liver specimen were isolated by an already established Biocoll/Percoll separation method and subsequently magnetic cell separation (MACS). Further assessment of purinergic receptors was performed by PCR analysis. Hepatic and blood derived NK cells were analysed by FACS. In a microfluidically supported liver biochip, comprising human cells, isolated NK cells were inserted and investigated under static or perfused condition for 48h.
Results: NK cells were successfully isolated with high purity from blood and liver specimen assessed by FACS analysis. In hepatic NK cells several purinergic receptors were identified (P2X4, P2X5, P2X7, P2Y1, P2Y4, P2Y8, P2Y13). After 48h under static and perfused conditions NK cells are still detectable within the biochip, without changes regarding CD16 and TRAIL status.
Conclusions: NK cells were successfully isolated and inserted into the existing microfluidically perfused liver biochip. NK cells attached and remained in the perfused in vitro model of the liver sinusoid. Several purinergic receptors were detectable on isolated hepatic NK cells. Further experiments to evaluate the influence of purinergic signaling are planned.

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Infection 2017

Role of Autophagic Lysosome Reformation (ALR) during inflammatory stress

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Introduction: Autophagy is an evolutionarily conserved lysosome-dependent degradation pathway, which can be induced by extrinsic and intrinsic stressors in living systems to adapt to fluctuating environmental conditions. In the context of inflammatory stress autophagy contributes to the elimination of invading pathogens, the regulation of innate and adaptive immune mechanisms, and regulation of inflammasome activity as well as tissue damage repair. Recently it was shown that lysosomes can be recycled from autophagy, a process also known as autophagic lysosome reformation (ALR). Thus ALR contributes to the replenishment of lysosomes that are available for fusion with autophagosomes in situations of increased autophagic turnover for instance during inflammatory stress or sepsis. Previously, our group and others could show that ALR is impaired in cells devoid of Spatacsin (SPG11) or Spastizin (SPG15).
Objectives: We aim to identify the effects of inflammatory stress on the lysosomal system, autophagy and ALR. For this purpose we will assess how different inflammatory mediators impact on the lysosomal system and in-vitro infection models.
Methods: Bone marrow derived macrophages (BMDMs) were isolated from wild-type mice and Spatacsin (Spg11−/−) knockout mice. BMDMs and Mouse Embryonic Fibroblasts (MEFs) were treated with 200 ng/ml of LPS for 14 h then fixed and stained with p62 and Lamp1 antibodies. Images were taken with Leica confocal microscope and the quantification of autolysosomes and lysosomes was done with ImageJ. Wild-type MEFs and Spg11−/− MEFs were infected with *Staphylococcus aureus* and multiplication of infection (MOI) used was 10. After 4 h of infection, cells were visually assessed for viability and images were taken under Olympus CKX53 inverted microscope.
Results: Prolonged starvation of wild-type MEFs pretreated with LPS induced a significant increase in autolysosomes as compared to starved untreated MEFs, while the number of lysosomes was diminished ($n = 45$ cells per group from 3 independent experiments, $^{**}P < 0.01$). Similar results were obtained for BMDMs after LPS treatment as compared to control ($n = 45$ cells per group from 3 independent experiments, $^{***}P < 0.001$).
In-vitro infection of MEFs with *Staphylococcus aureus* showed a marked decrease of cell viability in ALR deficient Spatacsin knockout (Spg11−/−) MEFs as compared to wild-type.
Conclusions: Significantly increased autolysosome numbers together with decreased lysosome number after LPS treatment is consistent with a defect of ALR in response to LPS. Suggesting that ALR is important for the defense of invading pathogens e.g. *S. aureus*, we observed a marked increase of cell death in an in-vitro infection model in cells with compromised ALR.

Clinical Sepsis Research: Diagnostics

001
Infection 2017

Room-temperature transpulmonary thermodilution (TPTD) with increased indicator volume of 20 ml compared to standard TPTD with 15 ml of iced saline

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Introduction: Due to the frequent haemodynamic instability in septic patients, haemodynamic monitoring including TPTD is frequently used to monitor cardiac index (CI), global end-diastolic volume index (GEDVI) and extravascular lung-water index (EVLWI). Current practice is to perform TPTD with iced saline boluses. However, this method can be cumbersome in daily routine, e.g. in the OR. A recent study (1) investigated TPTD using 15 ml room-temperature saline. The results of this study were not completely satisfactory for use in daily routine, though.
Objectives: This study aims to improve accuracy and precision of TPTD by using room-temperature saline by increasing the indicator volume.
Methods: 407 sets of TPTDs were performed in 60 patients monitored by the PiCCO-2 device. Each dataset consisted of four TPTDs, two with 20 ml room-temperature saline and two with 15 ml iced (4 °C) saline.
Results: Patients characteristics: 33 male, 27 female; APACHE II score 25 ± 8; height 171 ± 8 cm; weight 73 ± 14 kg; diagnosis 40% sepsis.
TPTD with 15 ml of iced saline vs. TPTD with 20 ml of room-temperature saline:
Compared to TPTD with iced saline the parameters derived from room-temperature injectate TPTD were slightly, but significantly higher for CI (3.96 ± 1.00 vs. 4.02 ± 1.06 l/min/m^2; $p < 0.001$), GEDVI (789 ± 159 vs. 793 ± 163 ml/m^2; $p = 0.013$) and EVLWI (11.1 ± 4.2 vs. 11.7 ± 4.5 ml/kg; $p < 0.001$). These differences resulted in acceptable values for mean bias and percentage error (PE) of 0.06 ± 0.35 l/min/m^2 and 17.4% for CI, 3.94 ± 77.60 ml/m^2 and 19.2% for GEDVI, 0.58 ± 1.20 ml/kg and 20.7% for EVLWI.
Influence of CVC position: Femoral CVC room-temperature indicator injection resulted in a slightly lower bias of GEDVI compared to jugular indicator injection (-4.97 ± 76.50 vs. 17.15 ± 77.55 ml/m^2; $p = 0.005$). The mean biases of CI (femoral: 0.03 ± 0.39 vs. jugular: 0.10 ± 0.29 l/min/m^2; $p = 0.110$) and EVLWI (femoral: 0.65 ± 1.22 vs. jugular: 0.49 ± 1.17 ml/kg; $p = 0.328$) did not differ for different CVC positions.
Comparison of 20 ml with 15 ml room-temperature injectate [original data from previous study (1)]:
CI (0.06 ± 0.35 vs. 0.15 ± 0.52 l/min/m^2; $p = 0.003$) and GEDVI (3.94 ± 77.60 vs. 30.47 ± 144.62 ml/m^2; $p < 0.001$) showed a significantly improved bias for 20 ml compared to 15 ml room-temperature injectate. Regarding EVLWI no difference could be shown (20 ml: 0.58 ± 1.20 vs. 15 ml: 0.59 ± 2.11 ml/kg; $p = 0.054$). PE was lower in all three variables for 20 ml injectate (CI: 17.4 vs. 21.9%, GEDVI: 19.2 vs. 29.2%, EVLWI: 20.7 vs. 29.3%).
Conclusions: TPTD with 20 ml of room-temperature saline showed acceptable bias and PE compared to TPTD with 15 ml of iced saline. TPTD with 20 ml of room-temperature saline showed better results than TPTD with 15 ml of room-temperature saline. Results so far suggest TPTD with 20 ml of room-temperature saline is applicable in practice.

References: (1) Huber, W., Kraski, T., Haller, B., Mair, S., Saugel, B., Beitz, A., Schmid, R. M., Malbrain, M. L. N. G. (2014). Room-temperature vs iced saline indicator injection for transpulmonary thermodilution. Journal of Critical Care, 29(6), 1133.e1137–1133.e1114. doi:10.1016/j.jcrc.2014.08.005

003
Infection 2017

Linezolid pharmacokinetics in liver failure: Exploring the maximal liver function capacity (LiMAx) test

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Introduction: Patients in the intensive care unit frequently require antibiotic treatment. Dose finding studies are usually performed in healthy volunteers or patients who are not critically ill. Extrapolation of the dosage recommendations to critically ill patients may not be reasonable due to pathophysiologic changes in this specific population. In addition, liver impairment possesses substantial challenges for dose selection in these patients.
Objectives: The aim of the present pilot study was to assess the novel maximal liver function capacity (LiMAx test) in comparison to conventional liver function markers as covariates of drug elimination in liver failure using linezolid as model drug. Therefor linezolid trough serum concentrations were prospectively evaluated in patients with or without liver dysfunction.
Methods: 28 patients with different degrees of liver failure were recruited and LiMAx test, plasma, dialysis and urine sampling was performed under linezolid steady-state therapy (600 mg BID). According to their LiMAx result at the respective measure point, parameters of each time point were matched into one of the following groups: (A) LiMAx <100 µg/kg/h (n = 11); (B) LiMAx 100–199 µg/kg/h (n = 14); (C) LiMAx 200–299 µg/kg/h (n = 9); (D) LiMAx ≥300 µg/kg/h (n = 17). NONMEM® was used for the pharmacometric analysis, in which the different clearance routes of linezolid were elucidated
Results: Linezolid pharmacokinetics were highly variable in patients with liver failure. The LiMAx score displayed the strongest association with CLnon-renal (=4.46•(WT/57.9)•(LiMAx/221.5)0.388 L/h), which reduced interindividual variability in CLnon-renal from 46.6 to 33.6%, thereby being superior over other common markers of liver function (INR, γ-GT, bilirubin, thrombocytes, ALT, AST). For LiMAx <100 µg/kg/h, 64% of linezolid trough concentrations were above the recommended trough concentration of 8 mg/L and the majority of patients with LiMAx >300 µg/kg/h revealed trough concentrations below 2 mg/L.
Conclusions: The study presents the first application of the LiMAx test to quantify the effect of liver dysfunction on the pharmacokinetics of linezolid. Thereby, the LiMAx test was superior over all other markers of liver dysfunction. Clinicians should be particularly watchful in patients with LiMAx <100 and >300 µg/kg/h and consider therapeutic drug monitoring with potential dose adaption of linezolid to avoid potential toxicity or therapeutic failure.
Acknowledgements: The study was funded by research grants of the Charité University Hospital, Berlin, Germany. No funds were obtained for payment of the authors. The authors would like to thank the team of the Surgical Intensive Care Unit of the Charité for supporting our study. We would also like to thank the team of the

"workgroup for the liver" for their valuable comments on planning and designing the study.

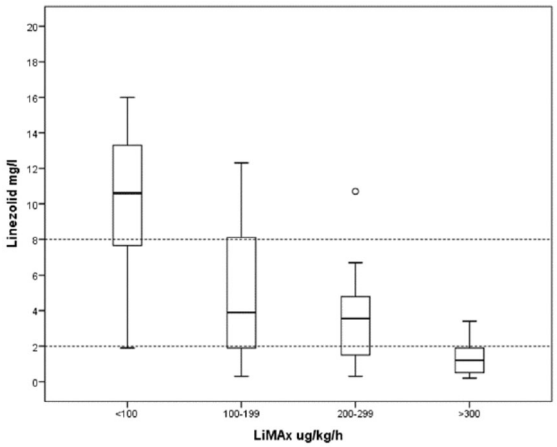

Figure 1: Linezolid serum level related to liver function measured with LiMAx test (normal range >315µg/kg/h)

004
Infection 2017

Pharmacokinetics of tigecycline in liver impairment: quantification of liver function with maximal liver function capacity test (LiMAx)

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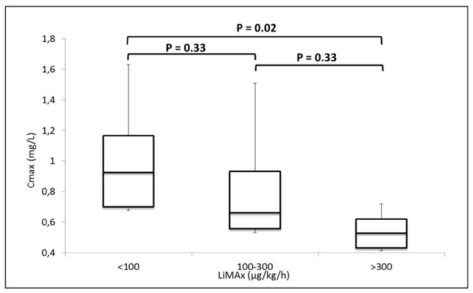
Introduction: Tigecycline (TGC), the first-in-class glycylcycline, is an important therapeutic option in critically ill patients due to its efficacy against (multiresistent) grampositive and gramnegative bacterias in the treatment of complicated intra-abdominal and complicated skin and skin-structure infections (1-2). Since TGC is metabolized and eliminated predominantly by the liver, critical illness induced liver failure may have a profound impact on the pharmacokinetic of TGC.
Objectives: The aim of the study was to establish a link between the degree of liver dysfunction and serum concentration of TGC over a quantitative measurement of liver function using the dynamic LiMAx test and a parallel determination of TGC serum concentration.
Methods: This prospective study includes 20 patients received TGC. Patients were treated with TGC by intermittent bolus administration of 100 or 50 mg every 12 h after a 100mg loading dose. TGC concentrations in serum and bile were obtained by collecting blood samples at 0.3, 2, 5, 8 and 11.5 h post dose for each patient after at least 36 h of therapy and were analyzed by means of a (HPLC method. Within the same day LiMAx test was carried out and routine blood parameters were measured. In addition we measured three patients in different dosage conditions: (1) directly after 100mg loading dose (2) in 100mg steady state (3) in 50mg steady state.
Results: Peak serum TGC concentrations (0.3 h after end of 50 mg dosage) were significantly higher in patients with severe liver failure (LiMAx <100µg/kg/h) than in patients with normal liver function (LiMAx >300µg/kg/h) (Fig 1). Pharmacokinetic curves showed higher values in severe liver failure at any measure point (Fig. 2). Serum concentrations in 100mg steady state were higher than in 50mg steady state, whilst serum levels after 100mg loading dose were lower than in 50mg steady state concentrations. TGC concentration in the bile duct were up to 50 times higher than in serum. TGC bile duct levels showed (Fig 3).
Conclusions: The study demonstrates a correlation between LiMAx test and TGC serum concentration. This qualifies LiMAx as a reliable index of the degree of liver function impairment and its drug-metabolizing ability, which is required in dosage adjustments in order to achieve maximum efficacy with minimum toxicity for an effective antibiotic therapy in the management of infection in critical illness.
Acknowledgement: The study was funded by research grants of the Charité University Hospital, Berlin, Germany. No funds were obtained for payment of the authors. The authors would like to thank the team of the Surgical Intensive Care Unit of the Charité for supporting our study. We would also like to thank the team of the "workgroup for the liver" for their valuable comments on planning and designing the study.

Tab 1: Baseline Characteristics

LiMAx-value (µg/h/kg)	<100 (n=7)	100-300 (n=18)	>300 (n=7)	P value [a]
Number of subjects (males/females)	(7/0)	(15/3)	(3/4)	0.07
Age (years)	73 (67-78)	68 (55-73)	61 (54-70)	0.03
Body mass index (kg/m2)	22.6 (21.2-24)	25 (24.2-30)	31.3 (24-46.4)	0.02
Creatinine clearance (mL/min)	62.6 (39.5-89)	43.9 (30.1-58.4)	32.4 (25.3-88.3)	0.81
Thrombocytes (cells/nL)	72 (47-111)	165 (101-295.3)	337 (201-462)	0.00
Bilirubin (mg/dL)	2.47 (1.78-6.47)	3.2 (0.64-4.63)	1.57 (0.85-2.24)	0.26
INR	2.11 (1.47-5.78)	1.47 (1.34-1.53)	1.29 (1.03-1.31)	0.00
GLDH (IU/L)	29.7 (3.1-669.4)	17.3 (5.18-78.7)	25.9 (9.6-39.9)	0.95

[a] p value presents the significance distribution between LiMAx value of <100 µg/h/kg and LiMAx value of >300 µg/h/kg

Fig 1: TGC peak serum level related to liver function measured with LiMAx test. (normal range >315µg/kg/h)



Bold lines indicate medians, box plots indicate 25th to 75th percentiles.

Fig 2: Mean TGC concentrations in serum between LiMax- groups after dose administration.

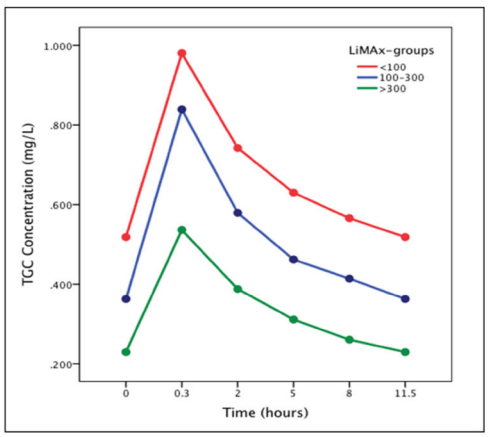
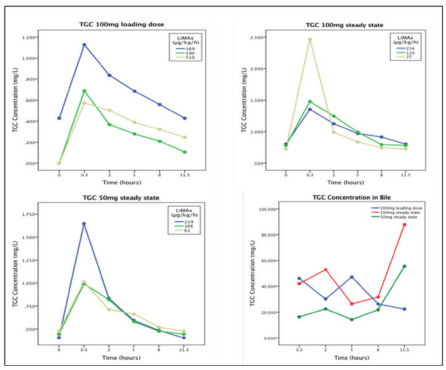


Fig 3: Individual TGC serum concentrations after 100mg loading dose, in 100mg and 50mg steady state and in bile obtained from three patients.



008
Infection 2017

Pathogen concentration integrated molecular analysis for SMARTDIAGNOS: the next generation sepsis diagnosis

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Introduction: Sepsis is a potential life threatening complication with the mortality rate ranging from 18–50% of the encountered cases. Each hour of delayed diagnosis and therapy will increase the risk of death by approximately 8%. In the European Union, overall mortality was 27–36% imposing an economical burden of approximately 7–21 B€ per year. The major limitation of sepsis diagnosis is the requirement of more than 48 h for the specific identification of causative pathogen due to the requirement of pathogen enrichment and culturing steps.
Objectives: Combination of pathogen pre-concentration with rapid molecular diagnosis is the main objective of the present work in order to overcome the present challenge of sepsis diagnosis: low pathogen concentration (5–10 CFU/mL blood) and to improve the current limit of detection by 1–2 orders of magnitude.
Methods: *Staphylococcus aureus* was selected as a model pathogen due to its predominance in sepsis complications. Anti-lipoteichoic acid antibody was immobilized on protein AG attached magnetic beads and used as capturing ligands. *Staphylococcus aureus* cells in a range ∼105 to 101 cfu in 1 mL of 1X PBS were incubated with the antibody immobilized beads (∼106 beads) at 37 °C for 30 min. The beads were washed and the captured pathogens were detected by PCR targeting Thermonuclease gene of *Staphylococcus aureus* . To mimic bloodstream infection, *Staphylococcus aureus* spiked volunteer human blood was used. The spiked blood was lysed with 2% saponin before incubating with antibody immobilized beads and analyzed as mentioned above.
Results: The Immuno-magnetic beads could efficiently concentrate *Staphylococcus aureus* at a low concentration of 102 cfu/mL from PBS and ∼103 cfu/mL from the spiked blood. Due to the highly viscous blood matrix, dilution of the blood had a direct influence on the immuno-capturing efficiency and dilution of 50% gave optimum results. The use of protein AG, as a bio-adaptor, provided unidirectional orientation to the immobilized antibodies thereby resulting in a long term antibody-bead conjugate stability with higher efficiency. Integration of a direct PCR using a phusion hot start DNA polymerase overcome the possible PCR inhibitors and excluded cumbersome DNA extraction steps thereby reducing the duration of analysis to less than 3 h.
Conclusions: The magnetic bead-based pathogen pre-concentration is efficient and versatile method to overcome the greatest challenges in sepsis diagnosis: low pathogen counts and complexity of blood matrix. Combination of the immuno-magnetic bead and the direct PCR reduces the sepsis diagnosis time significantly. This approach may lay a future platform technology for rapid detection of the pathogens causing sepsis.
Acknowledgement: This research financially supported by "SMARTDIAGNOS Next generation sepsis diagnosis technology" an EU Horizon-2020 project, Grant Agreement No.: 687697.

009
Infection 2017

Diagnostic value of midregional proAdrenomedullin (MR-proADM) and interleukin (IL)-17A for the detection of fungal infections in patients with septic shock—a prospective, single-center, observational study

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Introduction: Bacterial infections are the most common cause of sepsis. Although fungemia can only be observed in 3% of unselected septic patients, fungi are one of the most frequently isolated species in abdominal or respiratory tract specimens.
Objectives: The aims of this study were therefore twofold, (1.) to evaluate the prevalence of fungal colonization as well as infection in patients with septic shock and (2) to assess the diagnostic value of midregional proAdrenomedullin (MR-proADM) and interleukin (IL)-17A for the detection of fungal infections in these patients.
Methods: In total, 50 patients with septic shock were enrolled in this observational, prospective cohort study from 11/2013 to 01/2015. Fungal findings as well as corresponding plasma levels of MR-proADM and IL 17A-values were evaluated at 6 consecutive time points within 28 days after sepsis onset. Candida species (C. spec.) in the respiratory tract or in fluids from drainages were classified as colonization. Positive results in blood cultures, intraoperative swabs and Aspergillus species in deep respiratory tract specimens with accompanying pulmonary infiltrates were classified as infection.
Results: Within the 28-day observation period, 22% (n = 11) of patients suffered from a fungal infection. In contrast, 78% (n = 39) of patients were shown to be non-infected and either presented without any fungal findings (34%/n = 17) or were colonized with C. spec. in the different specimens (44%/n = 22).
In patients suffering from a fungal infection, plasma levels of MR-proADM were shown to be significantly increased at all timepoints in comparison to colonized patients as well as patients without any fungal findings. Accordingly, MR-proADM was shown to be a suitable tool for the identification of patients with a fungal infection as assessed by a receiver operating characteristic (ROC)-analyses (ROC-area under the curve (AUC) for patients with a fungal infection vs. non-infected patients e.g. at t0: 0.738; Cut-Off: 6,99nmol/l -> Sens. 0.727; 1-Spec. 0.333, t1: 0.755; Cut-Off: 8.53nmol/l -> Sens. 0.727; 1-Spec. 0.212, t2: 0.774; Cut-Off 5.10nmol/l -> Sens. 0.818; 1-Spec. 0.273, etc.).

IL-17A was also shown to be significantly increased in septic patients suffering from a fungal infection in comparison to septic patients with a fungal colonization or without any fungal findings within the first 7 days after sepsis onset. Therefore, IL-17A was also found to be a suitable tool for the identification of patients with a fungal infection as assessed by ROC-analysis (ROC-AUC for patients with a fungal infection vs. non-infected patients e.g. at t0: 0.714; Cut-Off 14.165pg/ml -> Sens. 0.818; 1-Spec. 0.323, t1: 0.776; Cut-Off: 14.22pg/ml -> Sens. 0.818; 1-Spec. 0.29, t2: 0.865 Cut-Off 15.00pg/ml -> Sens. 0.818; 1-Spec. 0.194, etc.).
Conclusions: Due to the diagnostic value for the detection of fungal infections in patients with septic shock, implementation of MR-proADM as well as IL-17A measurements in routine diagnostics should be taken into account.

010
Infection 2017

The clinical study of respiratory functional tests in abdominal sepsis

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Introduction: Multiple organ failure is a hallmark of abdominal sepsis (AS) being a leading immediate cause for sepsis-related mortality. Pulmonary dysfunction is a common sequel of sepsis, lungs are particularly vulnerable to the generalized inflammatory response, and sepsis is the cause of death in about 40% of all patients with acute respiratory failure. However, in about 50–60% cases it remains undiagnosed and untreated. Acute respiratory distress syndrome is the most common mechanism for pulmonary dysfunction, while SIRS, immunological reactions or direct pulmonary injury by microorganisms and toxins are rare. While many mechanisms of respiratory system involvement into sepsis pathogenesis are clear, the role of airways is not considered as a possible treatment target.
Objectives: The aim of the study is to determine changes of pulmonary function under AS to distinguish them from surgery-associated influences.
Methods: The study includes clinical observations of 7 AS patients (1st group), 9 patients who underwent uncomplicated minor elective surgery not related to abdominal pathologies (2nd group), and practically healthy individuals (3rd group—control). Computer assisted spirometry, simplified breathing retention tests, pO2 and pCO2 at rest and during oxygen test performed on 3rd day after surgery; contraindications and bioethics were strictly obeyed. Post-bronchodilator test was not performed.
Results: The max breath holding on inhalation phase in groups was as follows (sec): 12.85 ± 3.81 (group 1), 29.36 ± 1.24 (group 2) and 39.08 ± 3.65 (group 3), respectively ($p < 0.05$); max breath holding on exhalation phase was (sec): 8.93 ± 0.53 (1), 21.38 ± 2.84 (2) and 25.63 ± 3.73 (3), respectively ($p < 0.05$). pO2 at rest 53.64 ± 3.28 (1), 69.04 ± 5.02 (2), and 73.51 ± 2.67 (3), respectively; pCO2 at rest 30.63 ± 2.70 (1), 49.26 ± 3.07 (2), and 40.47 ± 1.68 (3), respectively. pO2 after pure oxygen supply—64.32 ± 1.94 (1), 70.37 ± 3.75 (2), and 75.76 ± 1.52 (3), respectively; pCO2 after pure oxygen supply—31.66 ± 2.58 (1), 41.26 ± 3.89 (2), and 38.35 ± 1.73 (3), respectively. Computed spirometry aborted in all patients of group 1 (AS group) due to very low figures (less than 6 s FVC test), discomfort and difficulties.
Conclusions: While this study has multiple limitations and possible errata, received data confirms existence of respiratory insufficiency in abdominal sepsis. Moreover, in addition to known mechanisms, possible involvement of airways is currently underestimated in treatment protocols. It is still unclear whether it is determined by anesthesia or immune misbalance, requiring further studies.

011
Infection 2017

The clinical study of respiratory functional tests in abdominal sepsis

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Introduction: Multiple organ failure is a hallmark of abdominal sepsis (AS) being a leading immediate cause for sepsis-related mortality. Pulmonary dysfunction is a common sequel of sepsis, lungs are particularly vulnerable to the generalized inflammatory response, and sepsis is the cause of death in about 40% of all patients with acute respiratory failure. However, in about 50–60% cases it remains undiagnosed and untreated. Acute respiratory distress syndrome is the most common mechanism for pulmonary dysfunction, while SIRS, immunological reactions or direct pulmonary injury by microorganisms and toxins are rare. While many mechanisms of respiratory system involvement into sepsis pathogenesis are clear, the role of airways is not considered as a possible treatment target.
Objectives: The aim of the study is to determine changes of pulmonary function under AS to distinguish them from surgery-associated influences.
Methods: The study includes clinical observations of 7 AS patients (1st group), 9 patients who underwent uncomplicated minor elective surgery not related to abdominal pathologies (2nd group), and practically healthy individuals (3rd group—control). Computer assisted spirometry, simplified breathing retention tests, pO2 and pCO2 at rest and during oxygen test performed on 3rd day after surgery; contraindications and bioethics were strictly obeyed. Post-bronchodilator test was not performed.
Results: The max breath holding on inhalation phase in groups was as follows (sec): 12.85 ± 3.81 (group 1), 29.36 ± 1.24 (group 2) and 39.08 ± 3.65 (group 3), respectively ($p < 0.05$); max breath holding on exhalation phase was (sec): 8.93 ± 0.53 (1), 21.38 ± 2.84 (2) and 25.63 ± 3.73 (3), respectively ($p < 0.05$). pO2 at rest 53.64 ± 3.28 (1), 69.04 ± 5.02 (2), and 73.51 ± 2.67 (3), respectively; pCO2 at rest 30.63 ± 2.70 (1), 49.26 ± 3.07 (2), and 40.47 ± 1.68 (3), respectively. pO2 after pure oxygen supply—64.32 ± 1.94 (1), 70.37 ± 3.75 (2), and 75.76 ± 1.52 (3), respectively; pCO2 after pure oxygen supply—31.66 ± 2.58 (1), 41.26 ± 3.89 (2), and 38.35 ± 1.73 (3), respectively. Computed spirometry aborted in all patients of group 1 (AS group) due to very low figures (less than 6 s FVC test), discomfort and difficulties.
Conclusions: While this study has multiple limitations and possible errata, received data confirms existence of respiratory insufficiency in abdominal sepsis. Moreover, in addition to known mechanisms, possible involvement of airways is currently underestimated in treatment protocols. It is still unclear whether it is determined by anesthesia or immune misbalance, requiring further studies.

013
Infection 2017

Changes of cardiovascular system functional state in abdominal sepsis

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Introduction: Cardiovascular system is a primary targeted organ in sepsis causing about half of all sepsis-related mortalities. Abdominal sepsis (AS) often caused by endotoxin producing microorganism with increased intra-abdominal pressure aggravates cardiovascular load by means of direct toxic influence and compartment syndrome development. Current understanding of cardiovascular changes in AS varies from 'hyperdynamic circulation' to cardiovascular failure and shock.
Objectives: Hemodynamic and perfusion disorders are significant burden, form pathogenesis and are found in almost all AS patients. In connection with the above, the purpose of the study was to determine changes of the functional state of the cardiovascular system in AS.
Methods: The study included 17 patients with AS (group I), 15 patients after elective surgery for uncomplicated anterior abdominal wall hernia and varicose veins of lower extremities (group II). The control (group III) formed by 21 healthy volunteer. Following hemodynamic parameters (systolic blood volume [SBV], minute blood volume [MBV], mean dynamic pressure [MDP], systemic peripheral vascular resistance [SVR], and autonomic nervous system regulatory parameters) for 2–3 days after surgery were studied. The study approved by the bioethics commission.
Results: Obtained data (table) confirms that any surgery significantly impact parameters of central and peripheral hemodynamics. Group 2 changes indicate the active involvement of compensatory-adaptive mechanisms and stimulation of the cardiovascular system; such changes may not be considered pathological. In AS patients HR grew significantly while SVR dropped ($p < 0.05$). Influence of the sympathetic autonomic nervous system in AS increases dramatically; the Kérdö index in 1 group patients was (+) 42.37 ± 7.61, while for the 2 and 3 groups it was moderately negative (−) 0.024 ± 0.006 and (−) 0.16 ± 0.03, respectively. Hildebrandt's coefficient practically unchanged in AS (4.32 ± 0.69 vs. 4.55 ± 0.71 in 2 group, and 4.10 ± 0.32 in control, $p > 0.05$). Accordingly, the value of changes of the autonomic nervous regulation of intersystem interaction between the respiratory and cardiovascular systems in the pathogenesis of AS is insignificant.
Conclusions: Functional failure of the cardiovascular system emphasizes abdominal sepsis of any severity. Changes of the functional state of the cardiovascular system not only worsen the prognosis, but also significantly burden the regenerative processes in the peritoneal cavity and the healing of surgical wounds, increasing the risk of postoperative complications.

Indicators of systemic hemodynamics in AS patients

Variables	AS patients (I group) n=17	(II group) n=15	Control (III group) n=21
$MBV_{predicted}$(l/min)	4.26±0.13	4.47±0.16	4.34±0.09
MBV_{fact}(l/min)	8.84±0.47*#	5.17±0.42*	5.53±0.41
SVR (dyn·s/cm^5)	1172.0±95.11*#	1988.52±184.61*	1666.43±60.90
HR (b/min)	101.62±8.91*#	78.63±4.29*	68.51±3.86
SAP (mmHg)	96.81±3.75*#	123.20±5.82	119.60±4.43
DAP (mmHg)	58.31±4.09*#	83.91±2.07	79.63±8.22
PAT (mmHg)	32.40±4.72#	42.26±5.58	40.28±6.31

* – $p<0.05$ between control and study group;
# – $p<0.05$ between study groups.

022
Infection 2017

Longitudinal evaluation of plasma concentrations of presepsin and clinical used biomarkers for infection in patients after severe trauma

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Introduction: Due to better bleeding control, the high mortality of patients after multiple trauma is based on the higher risk of the development of subsequent infections. Thus, early onset of adequate therapy is of particular importance for patients' outcome after severe trauma. However, early rapid diagnosis is often masked by the consequences of sterile, damage-triggered immune response. Tissue hypoperfusion during shock and subsequent cell death as well as physically damaged cells and tissue result in the release of immunogenic damage-associated molecular patterns (DAMPs), capable of triggering a systemic inflammatory response syndrome (SIRS).
Objectives: Concerning the value for the monitoring of progress and therapy, biomarkers are seen as a supplemental approach, whereas the acute phase proteins C-reactive protein (CRP), procalcitonin (PCT) and the cytokine interleukin 6 (IL-6) are the most commonly used sepsis biomarkers in daily clinical routine. New biomarkers like soluble CD14 subtype (sCD14-ST, presepsin) and clot lysis index after 60 min (CLI 60) are described to indicate patients with acute septic diseases. While an increase of CRP, PCT and IL-6 may be caused by non-infectious reasons, an increase of presepsin was proposed to indicate a more specific infectious origin among different cohorts of patients. Furthermore, Adamzik et al. proved a significantly increased CLI60 in septic patients compared to postoperative patients and healthy subjects. This study aimed to analyze the course of presepsin and CLI60 compared to clinically used biomarkers of acute infections (CRP, PCT, IL-6) in a cohort of patients after severe trauma.
Methods: Between January 2015 to February 2016, we included 50 patients with severe trauma (Injury Severity Score >16). They were observed for seven consecutive days after ICU admission and screened for clinical routine data, signs of infection, and inflammatory biomarkers CLI60, sCD14-ST (presepsin), CRP, PCT and IL-6.
Results: Regarding the well establishes biomarkers CRP and PCT, we observed trauma-associated alterations, which were not correlated to the clinical development of SIRS (PCT: no-SIRS vs. SIRS $p = 0.50$). Presepsin was elevated at ICU admission. Elevation of Presepsin, IL-6 and CLI60 in the clinical course correlated with the development of SIRS (presepsin: no-SIRS vs. SIRS $p = 0.03$; IL-6: no-SIRS vs. SIRS $p = 0.03$; CLI60: no-SIRS vs. SIRS $p = 0.03$).
Conclusions: Our study systematically investigates both the kinetic of clinically established biomarkers of inflammation and infection as well as presepsin and CLI for after trauma. Both markers were proven to be not affected by the early immune reaction after trauma as well as over the next seven days, making them a valuable option as biomarkers for infection in this clinical setting worthy of further evaluation.

025
Infection 2017

Optimal ferritin value to diagnose hemophagocytic lymphohistiocytosis in intensive care units

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Introduction: Hyperferritinemia is one of the diagnostic markers of hemophagocytic lymphohistiocytosis (HLH), a rare life-threatening hyperinflammatory syndrome with fatal outcome. The HLH-2004 diagnostic criteria comprise a threshold of ferritin ≥500 µg/L, which, however, is at low sensitivity and specificity in adults.
Objectives: Therefore, our aim is to determine the most predictive ferritin value in adult HLH patients in the ICU.
Methods: This retrospective analysis approved by the ethics committee (EA1/176/16) was performed at the university hospital Charité-Universitätsmedizin Berlin, Campus Charité Mitte and Campus Virchow-Klinikum, Germany. We included 258 patients of at least 18 years old and hyperferritinemia according to the HLH-2004 diagnostic criteria, who were admitted to our ICUs between 2006 and 2014. If more than one ferritin value was available, we analyzed the highest. Statistical analyses were performed using non-parametric statistical tests, logistic regressions and ROC analyses.
Results: 9 out of the 258 patients were suffering from adult HLH. 73 out of the 258 patients died (28.3%). Ferritin was significant higher in adult HLH patients compared to non-HLH patients (median 1,266 vs. 15,920 µg/L; $p < 0.001$). This association remained significant after adjustment for age and gender ($p = 0.002$). ROC analyses revealed 100.0% sensitivity and 83.1% specificity for a ferritin of 3,095 µg/L (AUC 94.6%, $p < 0.001$, CI 0.901–0.992).
Conclusions: A ferritin value of 3,095 µg/L was best predictive for ICU patients suffering from adult HLH in our cohort. Inclusion of ferritin into the routine lab-panel in critically ill patients is warranted.

034
Infection 2017

AlertsNet 2.0—a Thuringia-wide population-based surveillance of bloodstream infections

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Introduction: Blood culture (BC) testing is recommended as a standard of care in international sepsis guidelines and has been shown to reduce intensive care unit stay, antibiotic use, and costs in hospitalized patients. The Thuringian registry AlertsNet (www.alertsnet.org), started in 2014, aims at quality assurance in BC diagnostics in the whole German federal state of Thuringia and connects hospitals and microbiological laboratories within an electronic registry for immediate integration and evaluation of BC findings. Population-based data are aspired within the next years.
Objectives: Data of the second turn of funding are presented.
Methods: Data from 21 hospitals with 8 associated labs were included in this analysis. Microbiological data of all BCs taken in the participating hospitals as well as clinical data from patients with clinically relevant positive BCs collected from August 2015 to August 2016 were analyzed using standard measures of descriptive statistics.
Results: In total, 55,642 BC sets have been taken in the participating hospitals representing 16,461 patients. After excluding negative BCs (46,437; 83.5%) as well as contaminants (2111; 3.8%) and after excluding positive BCs of the same patient taken within 96 h, a total of 7094 clinically relevant BC sets (12.8%) were identified

representing 3752 patients of which 1540 (41%) clinical data sets were available. Of these 1540 patients, 40.8% had a nosocomial bloodstream infection, while the remaining 59.2% were community acquired. Disease severity within 96 h after BC sampling ranged from infection without organ dysfunction (61.7%) to sepsis (29.5%) and septic shock (18.2%). The overall hospital mortality rate was 20.6%. 55.4% of patients with infection without organ dysfunction died, 17.2% of patients with sepsis, and 8.8% of patients with septic shock. Most common sites of infection were urogenital (29.7%), respiratory (22.0%), and abdominal/gastrointestinal sites (17.8%).
In 3,752 patients, Enterobacteriaceae, *Staphylococcus aureus* , and Enterococcus spp. belonged to the most frequently isolated pathogens (>50%). The most commonly anti-infective drugs given after first BC sampling in 4,431 episodes of empirical antibiotic treatment were Piperacillin/Tazobactam, Ciprofloxacin, and Meropenem.
Conclusions: The Thuringia-wide registry AlertsNet provides data on BC positive patients with a wide clinical range of bloodstream infections. Distribution of pathogens and underlying foci resembled the experience of previous population-based studies in other countries.
Acknowledgement: AlertsNet was funded by the German Ministry of Health (BMG, Grant IIA5-2512FSB114) and by the Thuringian Ministry for Social Affairs, Health and Family (TMSFG). AlertsNet 2.0 is supported by the German Federal Ministry of Education and Research (BMBF, grant 01EO1502) and by the Thuringian Ministry for Social Affairs, Health Care, Women and Family (TMASGFF, grant 44-0793/29-1-37527/2015).

035
Infection 2017

Calprotectin and calgranulin C as novel biomarkers of bacterial infection

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Introduction: Calprotectin (S100A8/9 protein) and calgranulin C (S100A12 protein) are calcium-binding proteins belonging to a group of danger associated molecular patterns (DAMPs). These proteins are stored in human epithelial cells, keratinocytes, monocytes (Mo), and neutrophils; caprotectin comprises approximately 45% of neutrophil cytosol content. After neutrophil death or activation, calprotectin and calgranulin C are released to extracellular space. Elevated serum levels of caprotectin and calgranulin C were found in children with acute otitis media, and in adults with tuberculosis or septic shock. Although these studies indicate that calprotectin and calgranulin C have potential as biomarkers of infectious diseases, their serum levels have not been analyzed in adults with common infectious diseases.
Objectives: Therefore, the aim of our study was to analyze calprotectin and calgranulin C serum levels in adult patients with community-acquired infectious disease. We also evaluated effect of antimicrobial therapy on the kinetics of these proteins and association with clinical course, etiology and source of the infection.
Methods: Adult patients (age range 18–80 years) admitted to the department of infectious diseases with probable severe bacterial or viral infections were enrolled in this prospective study. Blood samples were collected on day 1, 3, 5 and 7 after the admission. Beside routine laboratory analyses of blood count and clinical chemistry serum levels of caprotectin and calgranulin C were measured using ELISA test (Biovendor, Brno, Czech Republic).
Results: We enrolled 43 patients with bacterial infection (median age 53 years), 15 patients with viral infection (median age 31) and 25 healthy controls (median age 53 years). The most common diagnosis in bacterial infection group was genitourinary tract infection (n = 18) followed by lower respiratory tract infection (n = 11) and gastrointestinal infection (n = 4). Calprotectin and calgranulin C serum levels were significantly higher ($p < 0.05$) in patients with bacterial infection in comparison to viral infection and healthy controls. Furthermore, both proteins decreased significantly ($p > 0.05$) during 7 days of antibiotic therapy, which was reflected by uneventful recovery.
Conclusions: Our results suggest calprotectin and calgranulin C serum levels as potential biomarkers of severe bacterial infection. Moreover, serial measurements of these proteins in the blood might be used for evaluation of the efficacy of antibiotic therapy.
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048
Infection 2017

Diagnostic and prognostic value of monocyte chemotactic protein 1 in patients with sepsis and septic shock

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Introduction: Sepsis and septic shock represent complex disease syndromes with a significant impact on patientś outcomes. Monocyte Chemotactic Protein 1 (MCP1) is a pro-inflammatory acute-phase protein, which has been rarely investigated in sepsis and septic shock.
Objectives: This study evaluates the diagnostic and prognostic value of MCP-1 in patients with sepsis or septic shock being within the first week of intensive care treatment.
Methods: Patients with sepsis and septic shock according to Sepsis-3 criteria were included, when treated on an internal intensive care unit (ICU). 60 controls with no evidence for sepsis/septic shock were also included. Blood samples were taken on day 1, 3 and 8 of ICU treatment. Biomarker measurements were performed by ELIS for MCP-1 (Quantikine® ELISA), as well as for PCT, IL-6 and C-reactive protein (CRP). All-cause mortality was followed up at 30 days and 6 months.
Results: Of a total of 136 patients, 43 (32%) suffered from sepsis and 93 (68%) suffered from septic shock. Highest MCP-1 levels were found in patients with septic shock (median = 973.6pg/ml, IQR 608.4–2646.3pg/ml), followed by sepsis (median = 498.2pg/ml, IQR 378.4–903.6pg/ml) and controls (median = 350.6pg/ml, IQR 295.7–495.16pg/ml) ($p = 0.001$). MCP-1 was significantly higher in non-survivors compared to survivors both at 30 days (non-survivors: n = 68, median = 1074.1pg/ml, IQR 535.9–2373.2 pg/ml; survivors n = 68, median = 663.7pg/ml, IQR 460–1204.3pg/ml; $p = 0.02$) and 6 months (non-survivors: n = 87, median = 946.9pg/ml, IQR 540.3–2619.5pg/ml; survivors: n = 49, median = 577.1pg/ml, IQR 388.8–1136.2pg/ml; $p = 0.047$). Patients with MCP-1 levels within the 4th quartile were up to 3 times more likely to die within 6 months compared to patients with lower levels (HR = 2.1–3.3).

Conclusions: Levels of MCP-1 may discriminate sepsis and septic shock according to new sepsis-3 criteria and may provide prognostic value both for short- and mid-term all-cause mortality in these patients.

054
Infection 2017

Blood cultures at sepsis onset—guideline compliance and its impact on pathogen detection in a German cohort

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Introduction: Current guidelines recommend drawing at least two sets of blood cultures (BC) before starting antimicrobial therapy (AT) in patients with suspected sepsis or septic shock (1).
Objectives: To determine factors associated with pathogen detection from BC and with guideline compliance regarding BC.
Methods: We conducted a four year multicenter cluster randomized quality improvement study aiming at shorter time to AT and better BC diagnostics including ICU patients with sepsis (all with organ dysfunction) (2). The number of BC sets taken before or after the initiation of AT was recorded and results were classified on site as positive, contaminated or negative. Chi Square test was used to examine categorical variables.
Results: Of 6561 patients 2687 (41%) had at least two sets of BC drawn before starting AT (fully compliant), 2608 (40%) had only one set drawn or BC were drawn after the start of AT (partly compliant) and 1266 (19%) patients had no BC drawn at sepsis onset (non-compliant). Compliance was highest in emergency departments and low in operating theaters (Figure 1, $p < 0.001$). The number of sets drawn ($p = 0.015$) and the timing of BC draw before or after the start of AT ($p < 0.001$) were significantly associated with pathogen detection rates (Figure 2).
In a binary logistic regression model, the number of sets (odds ratio 1.15 per set, 95% confidence interval 1.06–1.24), BC draw before AT (OR 2.24, 95% CI 1.96-2.55) and procalcitonin concentrations (OR 2.05 per 10-fold increase, 95% CI 1.89–2.23) were independently associated with pathogen detection from BC. The quality improvement efforts were associated with a significant improvement in the number of cases with at least two BC sets drawn (from 68 to 84%, $p < 0.001$), a slight reduction of patients without blood cultures drawn (from 21 to 18%) but with no improvement in the proportion of blood cultures drawn before starting AT (from 52% to 48%) ($p = 0.047$).
Conclusions: Blood cultures drawn at the onset of severe sepsis have a high rate of pathogen detection, especially when guideline recommendations are fully followed. However, compliance with recommendations is low and differs between areas of care. While we were able to increase the proportion of cases with at least 2 BC sets drawn, our quality improvement trial had no influence on the timing of BC draw. Further quality improvement trials should especially focus on this topic and on areas of care with low guideline compliance.
Reference: (1) Dellinger RP et al. Intensive Care Med 2013
(2) Bloos F et al. Intensive Care Med 2017
Acknowledgements: The study was funded by the German Federal Ministry of Education and Research via the integrated research and treatment center "Center for Sepsis Control and Care" (FKZ 01EO1002).

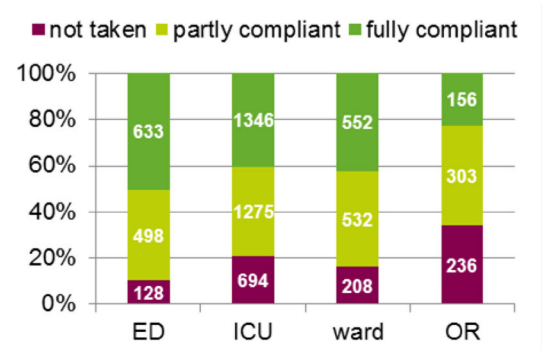

Fig. 1: BC compliance depending on place were first organ dysfunction was detected; emergency department or preclinical (ED), intensive care unit (ICU), operating room (OR)

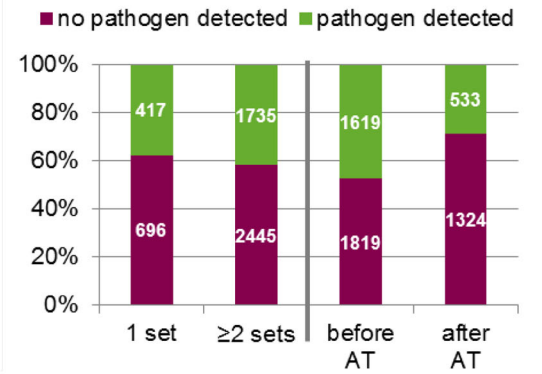

Fig. 2: BC results depending on number of sets drawn (p=0.015) or time of draw (p<0.001)

055
Infection 2017

Accelerating time to pathogen-adapted antibiotic treatment through culture-independent antimicrobial susceptibility testing in patients suffering from sepsis

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Introduction: Accurate and fast pathogen identification and consecutive antimicrobial susceptibility testing (AST) is of vital importance for patient outcome in patients suffering from sepsis.
Objectives: superiority of time-to-result generation by culture-independent AST when compared to gold standard.
Methods: The Accelerate Pheno^{TM} system is a new, fully automated, culture-independent diagnostic method for both pathogen identification (ID) and antimicrobial susceptibility testing (AST). We analyzed positive blood cultures from critically ill patients with new onset of sepsis according to the new sepsis guidelines, using both conventional

standard methods (VITEK, MALDI-TOF) and Accelerate Pheno™ system. ID/AST results of the Accelerate Pheno™ system were not reported to treating physicians as part of our internal evaluation process.

Results: Accelerate Pheno™ system correctly detected 74 pathogens [Gram-negative (GN) (n = 27), Gram-positive (GP) (n = 47)] straight out of 84 positive blood culture bottles. Gram-negative (GN) pathogens were identified as *E. coli* (n = 15; concordance rate 100%), *K. pneumonia* (n = 7; 71.4%), *S. marcescens* (n = 3; 100%), *E. cloacae* (n = 2; 50%), *P. mirabilis* (n = 1; 100%) and *P. aeruginosa* (n = 1; 33%). Gram-positive pathogens were identified as CNS (n = 24; 82.6%), *S. aureus* (n = 15; 88.2%), *E. faecium* (n = 6; 100%) and *E. faecalis* (n = 2; 100%). The Accelerate Pheno™ system generated a GN-AST result in 70.4% (19 of 27 samples) and a GP-AST result in 61.7% (29 of 47 samples) when compared to routine AST. Growth control, analysis and mechanical failure led to reduced results in comparison to conventional ID/AST. Accelerate PhenoTM delivered correct MIC-results for most of the panel antibiotics [e.g. meropenem: 83.3%, gentamicin: 88.9%, ertapenem: 100%].

Conclusions: The use of the Accelerate Pheno™ system significantly improved time-to-ID/AST and would have led to a reduced time-to-treatment in patients suffering from sepsis if results would have been reported. The system currently has some weakness in the detection of polymicrobial and streptococcal infections but due to the short hands-on-time, culture-independence and fast generation of results, it represents a promising new diagnostic method for the consecutive antibiotic treatment of septic patients.

058
Infection 2017

Two characteristic cytokine secretion and monocyte surface marker expression patterns are associated with the clinical outcome in critically ill patients with *Pseudomonas aeruginosa* induced sepsis

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Introduction: Critically ill patients with sepsis due to *Pseudomonas aeruginosa* (PSA) infections often reveal a prolonged stay and worse outcome.

Objectives: The present study was performed to find out whether these patients on a surgical intensive care unit with sepsis due to PSA with beneficial and worse outcome differ in serum cytokine concentrations and ex-vivo secretion, and in monocyte intracellular and surface receptor expression.

Methods: IL-8 serum concentrations and ex vivo secretion after LPS stimulation were determined by ELISA. Surface markers CD163 (hemoglobin scavenger receptor; clearance of hemoglobin, adhesion to endothelial cells, tolerance induction, tissue regeneration; soluble form: antiinflammation), CD206 (receptor for mannose on surface of microorganisms), CXCR1 (IL-8α chemokine receptor), CXCR2 (IL-8β chemokine receptor) and IFN-γ receptor as well as intracellular levels of IFN-γ of monocytes of patients with PSA sepsis and of healthy controls were analyzed by flow cytometry.

Results: 22 healthy controls and 20 surgical patients with sepsis/septic shock with underlying PSA infections were monitored. Compared to patients with low severity of disease SAPS II scores, patients with high SAPS II scores revealed significantly higher serum concentrations of TNF-α ($p < 0.05$) and sCD163 ($p < 0.01$), but lower concentrations of IL-8 ($p < 0.05$). In line with high IL-8 or low IL-8 concentrations in serum (965 ± 139 vs. 232 ± 15 pg/ml) as well as in culture after LPS stimulation (2838 ± 259 vs. 1097 ± 356 pg/ml), expression of surface markers IFN-γ, CXCR1, CXCR2 and CD163 was high or low, respectively, however, were always above those of the healthy control group ($p < 0.05$). In contrast, CD206 expression was higher on IL-8 low monocytes (3657 ± 279 MFI) than on IL-8 high cells (17 ± 6 MFI) ($p < 0.001$). The IL-8 low group manifested markedly higher severity of disease (SAPS II 34 ± 8) than the IL-8 high group (SAPS II 17 ± 6) ($p < 0.001$), and worse outcome.

Conclusions: Patients with sepsis due to PSA, with low TNF-α and sCD163 but high IL-8 serum concntrations, and a IFN-γhi, CXCR1hi, CXCR2hi, CD163hi, CD206lo monocyte marker profile are expected to have low severity of disease (SAPS II) and take a beneficial course. On the other hand, high TNF-α and sCD163 but low IL-8 serum concentrations, and a IFN-γlo, CXCR1lo, CXCR2lo, CD163lo, CD206hi marker pattern of monocytes may indicate patients with sepsis due to PSA with high severity of disaese and worse clinical outcome.

069
Infection 2017

Procalcitonin levels to predict blood culture results in patients with suspected infections

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Introduction: Blood cultures (BC) are the gold standard for detection of bacteremia in patients with suspected infections. However, a positive detection succeeds only in a limited number of patients. To predict BC results several studies found Procalcitonin (PCT) as one possible biomarker in patients with infections and suspected infections presenting in the Emergency Department (ED). (Laukemann et al. 2015)1.

Objectives: To predict BC results by PCT-levels in intensive care patients with suspected infections.

Methods: In a prospective clinical observational study (bedside vs. standard microbiological BC Diagnostics - BEMIDIA, ClinicalTrials.gov Identifier: NCT03000049) all consecutive ICU patients with clinical suspicion of infection and indication for blood culture sampling were included between November 2016 and April 2017.

Samples for three BC pairs and Point-of-Care (POC) –PCT measurement were taken simultaneously. BCs were incubated in an automated detection system (BD BACTEC™ blood culture system, BD Diagnostic Systems, Sparks, MD, USA). Pathogen identification was performed according to routine laboratory procedures. PCT-measurement was performed using a point-of-care device (Samsung IB B·R·A·H·M·S PCT, C&T Corporation, Seoul, South Korea).

Patients were classified into two groups according to their PCT levels <0.1 and >0.1 ng/ml.

Results: 121 patients with PCT measurments were included in this analysis. 28 patients (23%) of the samples resulted in a positive blood culture and a microbiological pathogen detection. 81 patients were treated with anti-infectives at the time of blood sampling. 18 patients (15%) had a PCT level less than 0.1 ng/ml. In these patients 11% of blood cultures were positive (95%Confidence Interval (CI)

0.02–0.36). 103 patients had a PCT level > 0.1 ng/ml while 25% BC (95% CI 0.17–0.35) were positive. Sensitivity and specificity of a cut-off by 0,1ng/ml for the POC-PCT-measurement were 93% (95% CI 0.75–0.98) and 17% (95% CI 0.10–0.27), respectively. The positive predictive value (PPV) of a cut-off by 0.1 ng/ml for blood cultures was 25% (95% CI 0.17–0.34) and the negative predictive value (NPV) 89% (95% CI 0.64–0.98).

Conclusions: Compared with patients in the ED (Lit.) where with a cut off of PCT <0.1 ng/ml NPV was 99.6% (PPV 12%) the results of this study in ICU patients showed a lesser NPV of 89%.

PCT levels of ICU patients therefore show minor advantage to predict positive BC. So BCs will be still required under these conditions. One possible explanation is that most patients were already treated with antibiotics at the time of blood sampling. Furthermore surgeries or malignomas can increase PCT levels.

Reference: 1 Laukemann, S. et al. Can We Reduce Negative Blood Cultures With Clinical Scores and Blood Markers ? Results From an Observational Cohort Study. 94, 1–10 (2015).

070
Infection 2017

Gender dependant transcriptome modulation in trauma patients: A longitudinal analysis

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Introduction: Studies in animals and humans have demonstrated that immune responses are highly gender-specific. Females have a more active baseline immune system. As a consequence, they exhibit a higher incidence of autoimmune diseases, a more robust response to vaccination, and a lower incidence of severe sepsis. However, the regulatory mechanisms behind, in particular in the critically ill are still unclear and appear complex.

Objectives: In this study, we aim to identify and synthesize evidence for the influence of gender on the transcriptome of immune cells in age and severity synchronized sepsis trauma patients throughout the course of the acute phase.

Methods: Publically available expression data was analyzed from a cohort of 75 men and 47 women age-matched (age > 18 and <50 years) critically ill trauma patients. Whole blood gene expression was measured from the time of hospitalization to 28 days at various time points. SOFA scores were used to synchronize the severity course of the patients. Transcription profiles were then mapped to the adjusted severity course and binned in such a way that acute, pre-acute and post-acute phase can be distinguished. Within each bin, transcription profiles of male versus female patients were compared to identify significantly distinct gene-sets. Identified gene-sets from all bins were then clustered into major groups of cellular processes using the k-means algorithm.

Results: A synchronization of samples according to their SOFA scores in time showed higher severity course in men in all bins (early, acute and post-acute). The maximum-SOFA score was significantly (p value <0.001) higher in men in spite of having similar injury scores as women at admission. In total, 321 gender-affected gene-sets were found modulated during the studied course of the illness. After eliminating small and heterogeneous clusters of gene-sets, we identified 8 large clusters representing the major molecular mechanisms that differ in regulation across gender. During the post-acute phase, women showed an immediate lymphocytic response. In turn, men exhibited higher gene regulation of mitochondrial functions and DNA damage response during the pre-acute and acute phase. The early up regulation of genes for respiration may be due to oxidative damage in men and a self-regulatory mechanism to compensate this but may need further therapeutic support, by e.g. antioxidants.

Conclusions: Our longitudinal gene expression analyses by adjusting age and severity scores have highlighted underlying gender-specific mechanisms. These altered mechanisms of immune- and recovery-responses may aid in understanding and delivering gender-specific point of care.

071
Infection 2017

Simple and rapid ciprofloxacin AST and MIC determination within 2 h using Raman spectroscopy

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Introduction: Antibiotic resistant bacteria, especially multi-resistant Gram negative bacteria (MRGN) gained an acute health care problem over the past years. Therefore, rapid antimicrobial susceptibility testing (AST) is urgently to avoid both, inappropriate under-treatment resulting in increased mortality in patients with sepsis and the unnecessary use of broad spectrum antibiotics fostering further spread of MRGN. Determination of the minimal inhibitory concentration (MIC) is recommended to tailor and optimize antibiotic selection and dosage. Here, we present a simple and fast spectroscopic procedure to identify antibiotic susceptibilities and furthermore to determine the MIC within 2 h.

Objectives: Our aim is to develop a simple and fast diagnostic tool based on Raman spectroscopy, allowing isolation of the pathogen from patients' material, identification of the pathogen and subsequent AST and MIC determination within a few hours.

Methods: Micro-Raman spectroscopy was applied to detect ciprofloxacin-induced changes in bacteria exposed to a range of several ciprofloxacin concentrations around the EUCAST breakpoint after only 90 min incubation time. The bacteria were captured directly in suspension using a dielectrophoresis chip, which is placed under the Raman microscope [1]. The spectroscopic MIC test was established using two *Escherichia coli* strains (AG100 and 3-AG100) and validated with 13 clinical isolates collected from patients with sepsis. Principal component analysis (PCA) was applied to define spectral ciprofloxacin effect marker bands. Classification as sensitive, intermediate or resistant was done according to EUCAST clinical breakpoints. To verify the MIC results, the spectral MICs were compared with MICs obtained by broth microdilution, Vitek-2 system and Etest.

Results: Raman spectra indicated a concentration-dependent killing effect induced by ciprofloxacin after 90 min of treatment. PCA identified two marker bands defined at 1460 and 1485 cm^{-1} that designate growth or inhibition. The ratios of the intensities of these

two bands show a clear concentration dependent effect and allow reading out the MIC at a defined threshold. The Raman-90-min AST results of the clinical *E. coli* isolates are in good agreement with the reference methods.
Conclusions: We present a Raman spectroscopy-based method for determination of the MIC within 2 h total analysis time using the clinical relevant example of *E. coli* and the fluoroquinolone drug ciprofloxacin. Only minimal sample preparation is required and short cultivation of 90 min is sufficient. These findings mark a major step towards the completion of a novel spectroscopy-based diagnostic tool.
References: [1] Schröder et al., Anal Chem, 2013, 85 (22), 10717–24
Acknowledgements: Financial support by the BMBF via the Integrated Research and Treatment Center "Center for Sepsis Control and Care" (FKZ 01EO1502) and the Carl Zeiss Foundation is highly acknowledged.

072
Infection 2017

Quantitative real-time analysis of the sublingual microvascular glycocalyx by emergency room and intensive care unit nurses—the GlycoNurse study

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Introduction: Deterioration of the endothelial glycocalyx (eGC), a protective carbohydrate-rich layer lining the luminal surface of the endothelium, plays a key role in vascular barrier dysfunction and eventually organ-failure in systemic inflammatory response syndrome and sepsis. Early detection of glycocalyx damage could thus become an important goal in critical care.
Objectives: This study was designed to determine the feasibility and reproducibility of quantitative, real-time glycocalyx measurements performed by trained nurses in the emergency room (ER) and intensive care unit (ICU).
Methods: The observational study included 70 patients admitted to the ER or ICU of a university hospital. The nurse in charge of the patient and a physician performed sublingual microcirculatory measurements using sidestream dark field (SDF) imaging. GlycoCheckTM software for automated data acquisition and analysis was used to analyze the perfused boundary region (PBR), an inverse parameter of endothelial glycocalyx dimensions in vessels with diameters of between 5 and 25 μm.
Results: There were no significant differences in the PBR values obtained by the nurses when compared to those reported by the physician (and which was regarded as the "gold standard" measurement). Intraclass correlation coefficient analysis showed excellent reproducibility between the nurses' and physician's PBRs (0.75 [(95% CI: 0.52–0.87]). The mean difference between the two PBRs (i.e., the bias) was 0.007 ± 0.25 μm. The nurses' PBR assessment had a 90% sensitivity (95% CI: 60–99%) and 90% specificity (95% CI: 80–93%) to identify a severely impaired glycocalyx.
Conclusions: ER and ICU nurses can reliably measure glycocalyx dimensions by non-invasive assessment of the PBR. This assessment could become part of standard monitoring and contribute to clinical decision-making and resuscitation protocols in clinical trials and daily practice.
Acknowledgement: AR designed the study, performed the measurements, analyzed the data, prepared the figures, and drafted the manuscript: AL, HV, JS, and HP contributed to the design of the study, discussed the findings and revised the manuscript; PK had the initial idea, supervised the study and contributed to the manuscript.

077
Infection 2017

Dramatic increase in the frequency of blood cultures and a significant decrease of bloodstream infections—an observational Study on 729 Intensive Care Units in Germany (2006, 2015)

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Introduction: Early and sufficient diagnostic to identify the causative pathogen is prerequisite for a timely therapy start and a targeted antibiotic treatment and essential for the patient outcome. Blood cultures are the most important diagnostic to detect a bloodstream infections.
Objectives: Aim of the study was to investigate the development of the frequency of blood cultures and the rate of primary bloodstream infections in a 10 year period (2006 to 2015) and to study the association between both parameters on intensive care units in Germany.
Methods: Intensive care units participating in the German hospital infection surveillance system (KISS) were included in the analysis. Bloodstream infection were defined according the Center for Disease Control and Prevention. Univariable and multivariable analysis were performed using generalized linear models (GLM).
Results: Overall, 2,427,921 patient days from 644,575 patients on 729 intensive care units were analyzed. On the 90 intensive care units reporting data 2006 and 2015, the frequency of blood cultures per 1000 patient days increased doubled from 57.8 (interquartile range [IQR] 29.8–101.2) to 128.2 (IQR 71.6–183.2). In the same time the mean bloodstream infection rate decreased by half from 2 (IQR 0–5; 2006) per 1000 patient days to 1 (IQR 0–3; 2015).
Nevertheless, taking a closer look at the intensive care units stratified by the frequency of blood cultures, wards with 200–249 compared to wards with 0–49 blood cultures per 1000 patient days, those with 200–249 had a 1.9 higher risk for bloodstream infections (incidence of bloodstream infections 0.44 versus 0.24).
Conclusions: In conclusion this investigation underlines the importance of a confidential feedback system within a surveillance system. We cannot just compare rates. Rates of bloodstream infections are significantly determined upon the frequency of blood culture. The risk of this surveillance bias must be taken always into account when surveillance data are used.

087
Infection 2017

The gut microbiota disturbances in ICU patients with nosocomial pneumonia

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Introduction: Individual gut microbiota is temporally stable, but under conditions of environmental changes in the intense-care unit (ICU), there are rapid dramatic disturbances and temporary dynamic changes in the microbial community composition. The analysis of 16S rRNA sequences in microbial populations using NGS gives a rapid overview of the microbial community diversity. Metabolic activity of microbes can be assessed by measurement of aromatic microbial metabolite levels (AMM)—which are associated with the severity and mortality of ICU patients. System response of the host organism to microbial load is assessed using biomarkers like procalcitonin (PCT) and presepsin (Ps).
Objectives: The purpose of our study is to assess the qualitative and quantitative parameters describing host reaction and gut microbes activity in patients with nosocomial pneumonia for better understanding pathophysiology of infection process in critically ill patients.
Methods: In a pilot prospective study, 5 patients with nosocomial pneumonia admitted to ICUs were observed on days 1, 3, 7–9 after the diagnosis of pneumonia. 16S rRNA metagenomic sequencing of fecal sample was performed using Ion PGM System, with Ion PGM Hi-Q^{TM} sequencing chemistry (318^{TM} Chip). DNA was extracted from endotracheal aspirate (EA) and fecal samples for the quantitative detection of nosocomial bacteria were analyzed using RT-PCR (IQ5, BIORAD); blood PCT and Ps were measured. After liquid-liquid extraction from serum and fecal samples, 9 phenylcarboxylic acids (AMM) were measured using GC-MS (Trace1310-ISQ, Thermo).
Results: The loss of species biodiversity of gut microbiota in ICU patients is reflected in a change of AMM profiles in fecal samples. According to metagenomic analysis, the major microbial families were Enterobacteriaceae, Enterococcaceae, Streptococcaceae, Staphylococcaceae, Bacteroidaceae (Fig. 1, Tab. 1). The following correlations with serum AMM were revealed: the total concentration of 9 AMM correlated with PCT (Spearman's correlation coefficient = 0.580), homovanillic acid - with PCT and Ps (rs = 0.810 and 0.709, respectively), phenyllactic acid - with presepsin (rs = 0.77), p-hydroxyphenylacetic acid - with total DNA of bacteria and DNA of Enterobacteriacea in EA (rs = 0.635 and 0.724, $p < 0.01$).
Conclusions: The biodiversity reduction in the gut microbiota is likely conduct to the change in the qualitative profile of AMM in the feces and their serum quantitative content that correlate with the host response (biomarkers level) in ICU patients with nosocomial pneumonia. Experiments with extended cohort will lead to a better understanding of the host-microbe interaction.
Supported by Russian Science Foundation Grant 15-15-00110

Figure 1. Temporal dynamics of relative abundance for 10 most abundant microbial species for each patient. White lines show alpha-diversity (measured using Shannon index, multiplied by 10 for visual convenience).

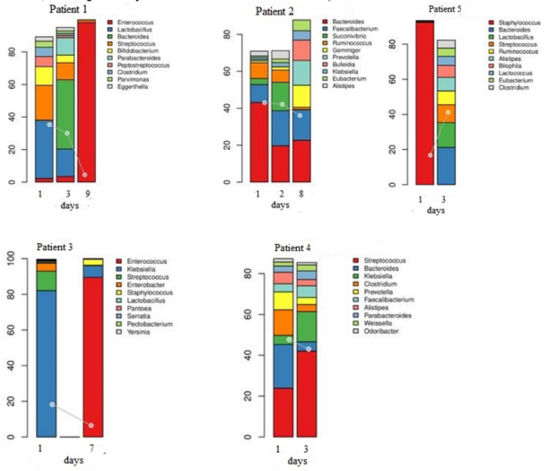

Table 1. The quantitative assessment of the bacterial load on the first day of nosocomial pneumonia diagnosis in the ICU patients (n = 5)

Pts №	APACHE II	SOFA	DNA EA, GE/ml		DNA feces, GE/ml		PCT, ng/ml	Ps, pg/ml	∑AMM, μM
			Total bacteria	Enterobacteriaceae	Total bacteria	Enterobacteriaceae			
1	15	3	1.3E+08	5.2E+07	1.4E+08	1.5E+08	0.395	1069	0.8
2	10	1	1.3E+09	7.2E+03	1.4E+08	9.6E+07	0.097	289	1.2
3	24	6	1.3E+06	4.4E+04	4.0E+08	2.3E+09	26,6	562	8.9
4†	30	5	1.2E+08	6.1E+07	1.5E+08	1.5E+08	1.84	5495	32.7
5†	31	8	6.1E+07	2.1E+03	3,0E+05	1,1E+04	2	nd	11.0

† - Fatal outcome, nd - no data;
** According to the microbiological examination, the following pathogens are identified in EA: Patient No. 1 - E. coli, carbapenem-resistant Klebsiella pneumonia, No. 2 - Moraxella spp., Streptococcus spp., No. 3 - multidrug resistant K. pneumonia, No. 4 - multidrug resistant K. pneumonia, Streptococcus spp., No. 5 - single gram-positive cocci*

088
Infection 2017

Changes in gastrointestinal microbiome patterns predict higher probability of postoperative complications

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Introduction: The development of postoperative complications is of great relevance in daily clinical practice and the gastrointestinal microbiome seems to play an important role by preventing pathogens from crossing the intestinal barrier.
Objectives: This study aims to examine changes in the gastrointestinal microbiome and evaluate its impact on the postoperative course following major abdominal surgery.
Methods: In total, 116 stool samples of 32 patients, undergoing pancreatic surgery, were analyzed via 16S-rRNA gene next-generation sequencing. One preoperative sample per patient was taken in order to determine the baseline microbiome without exposition to surgical stress and antibiotic use. At least two further samples within the first 10 days were taken in order to observe the longitudinal process. Whenever complications occurred, further samples were taken.
Results: We were able to allocate the samples to three different enterotypes (A, B and C) based on the structure of the microbiome. Enterotype B showed an increase of Akkermansia, Enterobacteriaceae and Bacteroidales as well as a decrease of Lachnospiraceae, Prevotella and Bacteroides. Patients being colonized by Enterotype B at least once during the observation period showed a significantly higher risk to suffer from postoperative complications (A vs B: odds ratio = 4.96, p value <0.01, B vs C: odds ratio = 0.34, p value = 0.019).
Conclusions: Within the presented investigation we were able to show for the first time, that there is a clear association between the structure of the gastrointestinal microbiome and the rate of postoperative complications.

089
Infection 2017

Acute fibrinolysis shutdown occurs early in septic shock and is associated with increased mortality

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Introduction: In the pathogenesis of sepsis, inflammation-induced changes in coagulation are essential for the host defense against infectious agents. However, an overwhelming procoagulatory response may lead to a situation in which coagulation itself contributes to the worsening of the disease. Acute fibrinolysis shutdown has recently been described to be an early biomarker for the identification of patients suffering from sepsis, although the underlying pathomechanisms, the specific temporal kinetics as well as its outcome relevance in humans remain to be determined.
Objectives: The aims of this study were therefore to evaluate the pro- as well as anticoagulatory responses in different inflammatory settings using whole blood thromboelastometry (TEM) in parallel with routine coagulation tests. A special focus was on the pathophysiology, kinetics as well as outcome relevance of fibrinolysis shutdown in patients suffering from septic shock.
Methods: In total, 90 patients (30 patients with septic shock, 30 surgical controls following major abdominal surgery and 30 healthy volunteers) were enrolled. Blood samples from patients with septic shock were collected at the time of sepsis diagnosis as well as 3, 6, 12, 24, 48 h and 7 d later. Samples from surgical patients were collected prior to the surgical procedure as well as 3, 6, 12, 24, 48 and 7d after the end of surgery. Blood samples from healthy volunteers were collected once. Besides TEM, routine coagulation tests were performed. Moreover, plasma concentrations of tissue plasminogen activator (total tPA, free tPA) and plasminogen activator inhibitor (PAI)-1 were measured using enzyme linked immunosorbent assays (ELISA).
Results: As assessed by significantly increased plasma levels of d-dimers, the coagulation system was shown to be activated in patients with septic shock in comparison to both control groups. Exceedingly, an inhibition of anticoagulatory mechanisms could be observed in septic patients as assessed by significantly decreased plasma concentrations of protein C, protein S and antithrombin (AT) III, which was paralleled by significantly increased plasma levels of thrombin-AT complexes. Moreover, fibrinolysis shut down occurred early in the course of the disease. Most interestingly, significant increases in total and free tPA in septic patients had no effect on thromboelastometrical lysis indices (LI30min, LI45min, LI60 min). Contrariwise, PAI-1 increased significantly already at sepsis onset, which was paralleled by significantly increased LIs in patients suffering from septic shock in comparison to both control groups. This effect persisted throughout the 7d observation period and was most pronounced in non-surviving septic patients.
Conclusions: As assessed by TEM, early inhibition of plasminogen activation led to acute fibrinolysis shut down with improved clot stability and was associated with increased mortality in patients suffering from septic shock.

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Infection 2017

The molecular face of hosts' blood transcriptome during early sepsis—insights from a large scale meta-analysis of microarray data

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Introduction: Sepsis is defined as a life-threatening condition, resulting from a dysregulated and harmful response of the hosts' immune system to infection. Apart from this, the (over-)compensating mechanisms counterbalancing the inflammatory response have been proven to render the host susceptible to further infections and increase mortality. To date, various disparate studies have characterized the pathophysiological complexity of sepsis with microarray-based transcriptome data of circulating immune cells, while frequently being restricted to a moderate number of patients in focus of particular infections or clinical diagnoses.
Objectives: Our study aims to unravel the heterogeneity of immune response in early sepsis and to elucidate the complexity of biological processes behind it.
Methods: Following stringent assessment of appropriate microarray-based studies, raw expression values from 14 selected data series provided from public high-throughput repositories were processed and annotated. In a subsequent conservative merging approach, a meta-dataset was created comprising 949 patients with sepsis and 135 healthy controls, being described by expression values for 28,544 unambiguously mapped gene symbols. Parametric empirical Bayesian methods were used for mandatory adjustments to accommodate for batch effects emerging in the combined dataset. The number of available genes was sequentially reduced to an informative subset of 5,000 genes with high variance. Residual expression data from septic patients were then passed to hierarchical clustering analysis.

Results: We identified two distinct clusters within the sepsis group (655 vs. 294 septic patients), in direct comparison exhibiting only moderate differences in gene expression. Comparing the both clusters individually to healthy controls yielded strong expression changes of genes involved in immune responses (T-cell receptor signaling, antigen presentation, inflammasome). Both comparisons found a similar set of regulated genes, with a stronger degree of dysregulation occurring in the larger cluster and implicating a loss of monocyte and T cell function, co-occurring with an activation of neutrophil granulocytes.
Conclusions: We propose a consistent - but in its extent varying - presence of immunosuppression, occurring as early in sepsis as its clinical manifestation. While certain cell types possess contradictory activation states, our finding underlines the urgent need for an early host-directed therapy of sepsis side-by-side with antibiotics.

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Infection 2017

Valuable LeukoDx64 score determination to follow antibiotic treatment during intensive care

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Introduction: Flow cytometry (FACS) is used to determine immune status of ICU patients by measuring expression rates of antigens like receptors on the surface of leukocyte subpopulations. Up to now FACS is a highly complex procedure with many steps and needs standardized protocols and specially trained technicians. In our laboratory FACS analysis of whole blood consists of nine steps and takes up to 3 h. The Accellix LeukoDx provides results for one marker, CD64 in 27 min and needs just one step by the user[1].
Objectives: We asked first whether the LeukoDx64 results correlated with flow cytometry and second, we asked whether CD64 is an earlier marker for infections and sepsis than plasma biomarkers, PC-T and CRP.
Methods: We compared the LeukoDx scores to the mean expression densities of CD64 on the surface of granulocytes, in addition to other surface markers related to leukocyte activation and HLA-DR. The Accellix LeukoDx cartridge performed the LeukoDX ratio measurements with 35µl of fresh EDTA-blood. Flow cytometry was performed with antibodies directed against isotype controls, CD11b, CD14, CD45, CD64, CD123, CD163. Analysis was done by a FACScalibur cytometer and Cellquest® (BD biosciences) and FloJo software, for lymphocytes, monocytes and granulocytes. Individual measurements as well as patients' courses were determined by FACS as well LeukoDx64 and were correlated using Graphpad prism vs. 7.0 for statistical evaluation.
Results: LeukoDx64 scores determined in 64 blood samples patients ranged between 0.59 and 22.39, and flow cytometrically determined expression densities on granulocytes ranged between 3.64 and 171. After matching each LeukoDx score with the conventional flow cytometry mean fluorescence intensities, Pearson correlation was calculated and gave an r-value of 0.9492 ($p = 0.0001$). In follow-up analyses, effective antibiotic treatment could be documented by a rapid decline of both, the LeukoDx64 score as well as mean fluorescence by conventional flow cytometry. Most importantly, the changes observed by CD64 quantification were significantly more sensitive than alterations in plasma biomarkers such as LBP (lipopolysaccharide binding protein) and PC-T.
Conclusions: We conclude that the LeukoDx64 score has a very high correlation with conventionally determined CD64 expression densities on granulocytes in patients with sepsis and septic shock before and during antibiotic treatment. Moreover, CD64 expression on granulocytes appears to be more sensitive to infection associated inflammation than LBP and PC-T.
Reference: [1]Sprung, C.L., Y. Sakr, J.L. Vincent, J.R. Le Gall, K. Reinhart, V.M. Ranieri, H. Gerlach, J. Fielden, C.B. Groba, and D. Payen. 2006. An evaluation of systemic inflammatory response syndrome signs in the Sepsis Occurrence In Acutely Ill Patients (SOAP) study. Intensive Care Med. 32:421–7.

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Infection 2017

Serum cholinesterase activity predicts length of the ICU stay following polytrauma

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Introduction: Severe traumatic injury (polytrauma) with resulting systemic inflammatory response is associated with prolonged intensive medical care and delayed patient recovery. The magnitude of the associated systemic inflammation plays an important role in the recovery following severe traumatic injury. Early detection of the systemic inflammatory response might prove helpful in the effective treatment of the polytrauma patients. Cholinergic activity has been shown to play an important role in the inflammatory response. Serum cholinesterase (butyrylcholinesterase; BChE) is the major acetylcholine hydrolyzing enzyme in blood. The activity of this enzyme has been shown to correlate with extent of the acute inflammatory response.
Objectives: Various biomarkers have been used in clinical and experimental practice to predict the patient outcome. However, a definitive prognostic tool for an early detection of the systemic inflammation and prediction of the patient outcome remains to be identified.
Here, we describe a correlation between the change in the BChE activity and the early systemic inflammatory response upon severe traumatic injury. Moreover, we assessed whether the BChE activity, when measured in patients at the hospital admission following polytrauma, could predict the patient outcome.
Methods: This observational clinical study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (Trial-Code No. S-391/2015) and the Ethics Committee of the Rheinland-Pfalz medical association, Mainz (Trial-Code No. 837.539.15/10307) and was conducted in the Trauma Centre Ludwigshafen, Germany. All patients or their legal designees gave written informed consent to the work. The BChE activity was measured by using point-of-care-test system (Securetec Detektions-Systeme AG, Neubiberg, Germany). In addition, levels of the routine inflammation biomarkers, i.e. C-reactive protein (CRP) and the white blood cell count (WBCC) were measured during the initial treatment period. Measurements were performed at the admission, followed by 12, 24 and 48-h time points. Injury Severity Score (ISS) was used to assess the trauma severity.
Results: The observed reduction in the BChE activity was in accordance with the change in the CRP concentration and the WBCC. The

BChE activity measured at the hospital admission negatively correlated with the length of the ICU stay in patients with polytrauma ($r = -0.5$, Spearman's rank correlation coefficient). A correlation between the ISS and the length of the ICU stay further confirmed our finding ($r = 0.7$, Spearman's rank correlation coefficient).

Conclusions: Our results suggest that the BCHE activity might be used as an early indicator for the magnitude of the systemic inflammatory response following polytrauma. Moreover, we show that the BChE activity, measured at the hospital admission, might predict the patient outcome and therefore prove useful in early identification of the high-risk patients.

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Infection 2017

Assessment of feasibility of using NLR as one of the markers of purulonecrotic complications development in patients with diabetic foot syndrome

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Introduction: Diabetes mellitus (DM) belongs to a group of metabolic disorders, the principal sign of which is high blood sugar level resulting from abnormal secretion and/or insulin efficiency. Diabetic foot syndrome (DFS) is one of the major complications of DM (5–10%) with a high rate of radical treatment-amputations. The lower limb amputation is performed in 0.2–4.8% of the DFS patients worldwide that is, on average, 20–30 times higher than in overall population [1].

Despite the progress in the diagnostics and treatment of DM, the DFS incidence rates are still high, being a significant indicator of inadequate control over the DM progression [2, 3]. Despite the achievements in modern medicine, i.e. advanced diagnostic techniques and continuous improvement of surgeries, the number of patients with purulonecrotic complications of DFS is growing each year [4].

Objectives: to assess the feasibility of using NLR as one of the markers of purulonecrotic complications in patients with diabetic foot syndrome.

Methods: The clinical material consisted of 118 patients who were divided into two groups. The first group included 59 patients with DFS and ulcerative soft tissue lesions. The second group comprised 59 patients with DFS without lesions. The average age of the patients was from 35 to 63 years old: man—43 (36.5%) and women—75 (63.5%). The research was conducted from March 2014 to November 2016. The patients of both groups participated in the following laboratory tests: complete blood count (WBC, neutrophil count, lymphocyte count, NLR), C-reactive protein (CRP) levels were recorded. The study has not included active infection, leukocytosis, malignancy, steroid use without any reason.

Results: At the hospital stage the study comprised a set of standard and additional research methods. The standard ones included general clinical research methods, assessment of foot lesion depth and of purulonecrotic lesion using the wound exudate study. The additional methods included the specification of purulonecrotic lesion depth and nature of the wound process.

As a result of the conducted studies, we received the following data (Table 1).

Variables	Group 1	Group 2	p
CRP	1.3 ± 1.4	0.5 ± 0.3	<0.001
WBC	10.1 ± 1.7	6.9 ± 1.9	0.236
Neutrophil percent (%)	65.3 ± 9.8	51.4 ± 8.8	<0.001
Lymphocyte percent (%)	22.6 ± 7.9	37.8 ± 8.9	<0.001
NLR	4.4 ± 3.8	1.5 ± 0.8	0.001

White blood cell counts, neutrophil counts, neutrophil% and the neutrophil lymphocyte ratio (NLR) were significantly higher in the first group compared to that in the second group.

NLR can be considered to be one of the markers of purulonecrotic complications development in patients with diabetic foot syndrome.

Conclusions: In this research, patients from both groups had analyzed NLR level as inflammatory marker. Determining NLR in the presence of high levels of diabetic foot ulcers is not a local inflammation alone but also showed that there is a systemic inflammatory response. In patients with diabetic foot other purulent complications of diabetes can be seen much more and using NLR has been suggested as a cheap and accessible inflammatory markers for developing and following of purulent complication.

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Infection 2017

Detection of *Staphylococcus aureus* Bacteremia by fully automated Multiplex PCR (Curetis Unyvero)

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Introduction: In order to be able to treat infections in a more focused and faster way, rapid and correct microbiological diagnostics are needed. The gold standard for the diagnosis of bacteremia is the classic microbiological blood culture (BC) diagnostic. This requires at least 24-48 h to get the final results. In comparison, the Curetis Unyvero System (Curetis GmbH, Holzgerlingen, Germany) potentially detects various microorganisms and resistance markers within 4.5 h.

Objectives: The present study was performed to verify the reliability of the results of the Curetis Unyvero System analysis compared to the gold standard of BC diagnosis.

Methods: In a prospective clinical trial (Bedside vs. Standard Microbiological BC Diagnostics - BEMIDIA, ClinicalTrials.gov Identifier: NCT03000049), 51 positive BC samples (196 samples with three BC pairs from 154 ICU-patients) were analyzed simultaneously by Curetis Unyvero System and by classic microbiology proceeding. Previously all BC samples were incubated in an automated detection system (BD BACTECTM blood culture system, BD Diagnostic Systems, Sparks, MD, USA).
The positive BC samples were lysed by the Unyvero L4 lysator within 30 min. Afterwards BC sample and Unyvero M1 Master Mix were inserted into the Blood Culture Cartridge and analyzed in the Unyvero A50 Analyzer.
With the cartridge 29 different gram-positive can be detected, 24 gram-negative and 8 fungi as well as Corynebacterium Spp., Mycobacterium Spp. and Propionibacterium Acnes. Simultaneously 16 different resistance markers were analyzed as well. The results of the automated multiplex PCR (Curetis Unyvero) were compared with the results of a classic microbiological proceeding.
Results: 51 (26.02%) BC incubated in BACTEC were positive for bacteremia and 17 of those (8.67%) for *Staphylococcus aureus* . 15 (7.65%) of the *Staphylococcus aureus* positive BC were identified in classical microbiology as well as in the Curetis Unyvero System. In one sample the Curetis Unyvero System found a Methicillin Resistant Staphylococcus Aureus (MRSA) and the resistance pattern mecA even though classical microbiology was negative for *Staphylococcus aureus* . There was no MRSA detected in any of the patients microbiology samples, not even in the routinely performed nasal MRSA-PCR screening. In a second sample we found *Staphylococcus aureus* with classical microbiology but not with Curetis Unyvero System.
Conclusions: Our first results show a correspondence with the results gathered through microbiological analysis of *Staphylococcus aureus* bacteremia within ICU-patients. More data are necessary to analyze the benefit of Curetis Unyvero System in patients with other bloodstream infections than *Staphylococcus aureus* bacteremia and to find the clinical benefit of this rapid germ detection in positive blood cultures.

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Serum cholinesterase activity predicts length of the ICU stay following polytrauma

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Introduction: Severe traumatic injury (polytrauma) with resulting systemic inflammatory response is associated with prolonged intensive medical care and delayed patient recovery. The magnitude of the associated systemic inflammation plays an important role in the recovery following severe traumatic injury. Early detection of the systemic inflammatory response might prove helpful in the effective treatment of the polytrauma patients. Cholinergic activity has been shown to play an important role in the inflammatory response. Serum cholinesterase (butyrylcholinesterase; BChE) is the major acetylcholine hydrolyzing enzyme in blood. The activity of this enzyme has been shown to correlate with extent of the acute inflammatory response.
Objectives: Various biomarkers have been used in clinical and experimental practice to predict the patient outcome. However, a definitive prognostic tool for an early detection of the systemic inflammation and prediction of the patient outcome remains to be identified.
Here, we describe a correlation between the change in the BChE activity and the early systemic inflammatory response upon severe traumatic injury. Moreover, we assessed whether the BChE activity, when measured in patients at the hospital admission following polytrauma, could predict the patient outcome.
Methods: This observational clinical study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (Trial-Code No. S-391/2015) and the Ethics Committee of the Rheinland-Pfalz medical association, Mainz (Trial-Code No. 837.539.15/10307) and was conducted in the Trauma Centre Ludwigshafen, Germany. All patients or their legal designees gave written informed consent to the work. The BChE activity was measured by using point-of-care-test system (Securetec Detektions-Systeme AG, Neubiberg, Germany). In addition, levels of the routine inflammation biomarkers, i.e. C-reactive protein (CRP) and the white blood cell count (WBCC) were measured during the initial treatment period. Measurements were performed at the admission, followed by 12, 24 and 48-h time points. Injury Severity Score (ISS) was used to assess the trauma severity.
Results: The observed reduction in the BChE activity was in accordance with the change in the CRP concentration and the WBCC. The BChE activity measured at the hospital admission negatively correlated with the length of the ICU stay in patients with polytrauma ($r = -0.5$, Spearman's rank correlation coefficient). A correlation between the ISS and the length of the ICU stay further confirmed our finding ($r = 0.7$, Spearman's rank correlation coefficient).
Conclusions: Our results suggest that the BCHE activity might be used as an early indicator for the magnitude of the systemic inflammatory response following polytrauma. Moreover, we show that the BChE activity, measured at the hospital admission, might predict the patient outcome and therefore prove useful in early identification of the high-risk patients.

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Early detection of ESBL by Accelerate Pheno™ system—a case report

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Introduction: To treat infections with multiresistant bacteria, rapid and correct microbiological diagnostics are needed. The gold standard for the diagnosis of bacteremia are the classic microbiological (CM) blood culture diagnostics. After positivity the CM cultivation needs 24–48 h for a final pathogen result. In comparison, the Accelerate Pheno™ system (APS, Accelerate Diagnostics, Inc., Tucson, Arizona, USA) detects various microorganisms, resistance patterns and minimal inhibitory concentration (MIC) within 6 h after positivity.
Objectives: To demonstrate a case of rapid detection of Extended Spectrum Beta-Lactamase (ESBL) Klebsiella pneumoniae in a positive blood culture (BC) with consequences of antimicrobial therapy.
Methods: In a prospective clinical trial (Bedside vs. Standard Microbiological BC Diagnostics - BEMIDIA, ClinicalTrials.gov

Identifier: NCT03000049), positive BC samples are analyzed simultaneously by APS and by CM. The BC samples were incubated in an automated detection system (BD BACTEC™ blood culture system, BD Diagnostic Systems, Sparks, MD, USA).
The positive BC sample were put in the APS. After the lysis of the samples in flowcells a gel electrofiltration follows. A current is applied so that interfering material will be absorbed leaving only the pathogens for analysis. A fully automated fluoresence in-situ hybridisation leads to the identification of the pathogens within 90 min. Detected bacteria is cultivated in a given concentration of antibiotics and time-lapse imagine detects the antibiotic susceptibility and MIC within 6 h.
Results: A male, 51 year old patient was transferred from a rehabilitation clinic to the hospital. 16 days later the patient showed a reduced vigilance, a high PCT of >10 ng/l and a positive urin status with >400 leukocytes/mikrolitre. BCs were taken and with the probable cause of urosepsis a calculated therapy with piperacillin-tazobactam was started, considering a past colonisation with *Pseudomonas aeruginosa*. The following day the BC became positive (09:30am, +0 h after positivity) and samples were sent to CM diagnostics and simultaneously the APS started. 90 min after positivity (11:03am, +1,5h) the APS identified Klebsiella pneumoniae. One and a half hour later (12:22 am, +3h) the CM result (MALDI-TOF mass spectrometry) came as well. Seven hours after positivity (04:21pm, +7h) the APS presented the resistogram identifying 3MRGN, ESBL and the therapy was adjusted to meropenem. The patient could be transferred the same day with improved vigilance. The final CM results arrived two days after positivity confirming the resistogram of the APS (08:00, +41,5h)
Conclusions: The APS can shorten the time to identify pathogens and to start an adequate therapy. Especially in infected patients with multiresistant bacteria the earlier detection is suitable to save lives, because of the faster adjusted antibiotic therapy.

Clinical Sepsis Research: Therapy

014
Infection 2017

The septic patient with new-onset atrial fibrillation—how to treat?

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Introduction: Atrial fibrillation (AF) is the most common arrhythmia which occurs during sepsis or septic shock and is promoted by systemic inflammation and atrial remodeling. Especially the occurrence of new-onset AF with no history of preexisting "typical" AF is associated with increased morbidity and mortality. It can further lead to hemodynamic failure and secondary thromboembolic complications such as stroke. Yet, there are no specific guidelines on treatment of septic patients with new-onset AF and its ideal management currently remains unclear.
Objectives: To investigate the state-of-the-art on anti-arrhythmic and stroke-preventive therapeutic strategies specifically for patients with new-onset AF during sepsis.
Methods: A systematic review of the literature was conducted on MEDLINE using various search terms. Abstracts of the listed publications were systematically analyzed and included if relevance and quality matched our inclusion criteria. We manually added current versions of major guidelines focusing on AF management to investigate their current treatment approaches for new-onset AF, AF during sepsis or AF due to other inflammatory causes.
Results: 1 meta-analysis, 3 reviews and 11 studies were included. AF management guidelines of the European Society of Cardiology, the American Heart Association and the American Association for Thoracic Surgery were investigated. The Surviving Sepsis Campaign guidelines for clinical sepsis management were also included.
The current standards determine that the necessity of immediate electric cardioversion is dependent on hemodynamic stability. Electrical cardioversion alone seems to be ineffective and should most likely be combined with amiodarone. Evidence on a pReference of rhythm or rate control in hemodynamically stable patients is poor. Systemic anticoagulation for stroke prevention is crucial but it seems that typical scoring systems such as CHA2DS2-VASc score fail to identify septic patients at risk. According to studies and guidelines, anticoagulation in new-onset AF - especially during and after cardioversion - should only be administered to patients at high risk for stroke.
Conclusions: While adequate antibiotic therapy and if necessary surgical intervention as a causal approach to eliminate the inflammatory mechanisms responsible for new-onset AF during sepsis form the basis of the treatment strategy, exact knowledge on specific treatment options of new-onset AF in the septic patient is lacking and further prospective treatment studies are required. Stroke-preventive anticoagulation should only be performed in patients at high thromboembolic risk, while the exact risk factors associated with stroke during sepsis are currently unknown. Even though there is no definite data on follow-up of patients with new-onset AF during sepsis, routine long-term cardiology examinations after such an episode are recommended.

015
Infection 2017

Death in ICU—an evaluation from Malawi

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Introduction: Information on survival rates in sub-Saharan African ICUs (outside the Republic of South Africa) is scarce. Even rarer are studies on longterm developments of survival and mortality rates. These data are needed as basis to study death rates of sepsis.
Objectives: To investigate the survival rates on our ICU in one of the poorest countries on earth over a period of around 10 years.
Methods: Audits, studies, publications, presentations and hospital data from the largest Malawian hospital and ítś ICU were retrieved, collected and analysed for the years 2004 to 2014.
Results: Early small studies from 2004 and 2005 showed mortality rates of 54% and 41%. Our first own survey from October 2006 to October 2007 showed a mortality of 40%. In the second from September 2009 to August 2010 we found a mortality on our ICU of 37.9%, followed by the third during the period from September 2010 to August 2011 with 34%. From June 2011 to June 2013 this could be reduced in the fourth to 29.8%. Our fifth and last study from September 2013 to mid-November 2014 showed a further reduction to 23.6%.
Our hospital budget was reduced (2007 to 2014) from around 1.7 million to 1.37 million US $ by 19.4%. The pressure on our ICU was further pronounced due to the admission of more HIV-reactive patients with and without sepsis thanks to the change of AIDS from an acutely deadly disease towards a treatable condition with the availability of antiretroviral substances payed for by the Global Fund.

Nevertheless from our first to our last study the above numbers represent a reduction of 59% in eight years.
Conclusions: Even in ressource poor countries it is possible- despite rising medical and economical constraints- to reduce the mortality in the intensive care units to a level which is finally acceptable.

019
Infection 2017

Parallel treatment with anti-infectious drugs in an African ICU

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Introduction: Information on interactions and sideeffects of multiple anti-infectious (a-i) therapy in Africa is rare. To shed light on this we performed an audit in the ICU of Malawis largest hospital.
Objectives: To find basic information on multiple a-i-therapy in an African critical care unit.
Methods: From 1.1.-29.2.2014 we surveyed retrospectively in our ICU numbers and types of a-i substances, mortality, signs of infection and social challenges related to HIV-therapy during the first day of admission in our mainly surgical ICU.
Results: Overall 53 patients received 119 anti-infectious drugs (2.24 substances per patient). Patients who died received 3 substances per person. Only 2 patients did not receive any a-i drug (3.7%) and 6 patients (9.4%) received 5 or more a-i substances parallel. 1 of these multi-drug receivers died (16.6%). Overall mortality was 11.3%. Both patients without a-i drugs survived. Ceftriaxon was most widely used (83%) followed by Metronidazol (60.4%).
In 28 cases an infection was suspected or proven (52.8%). Mean temperature was 36.9 degrees Celsius. Four patients (7.4%) were treated with antimalarial, -tuberculotic or -retroviral drugs (ARVs) independant of the reason for admission. Two of them died (50%).
We found these data underestimating the number of patients on parallel treatment because the surgical population in our hospital is 40% HIV-reactive and 50% of Malawians in need of ARVs are treated (no discrimination against the admission of reactive surgical patients). Different medical opinions on when a patient should receive ARVs in ICU and social reasons like the spouse who brings ARVs secretly at night for her husband to avoid stigma were discovered.
Conclusions: Too many a-i substances are given without good reasons. A difficult clientel are patients receiving already anti-tuberculotic or -retroviral therapy before they are acutely treated for infections or sepsis.

020
Infection 2017

Daily goals for septic and non-septic patients in a tropical intensive care unit and causes for failure to achieve them

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Introduction: Queens Hospital is the largest referal center in Malawi (>250,000 in-patients/year). Our 4–5 intensive care beds (all ages and departments) are a precious resource for all patients, especially including our many septic patients. Unnecessary challenges in organization, communication, coordination, interaction or problem-awareness, which might lead to prolonged patient stays need to be reduced.
Objectives: To assess documentation of daily goals during our ICU ward rounds for septic and non-septic patients, the percentage of their realization, the analysis of reasons inside and outside ICU leading to a failure to achieve our goals. To assess the effectiveness of a checklist for following audits.
Methods: We conducted an observational, descriptive, prospective clinical audit and a re-audit in our ICU during 20 ward rounds in 20 days from 8th February 2016 to 4th March 2016. The audit-team was part of the morning ICU ward-rounds, took notes and engaged the ICU team during handover at 4 pm on why agreed goals from the morning were not achieved. The re-audit used an adopted checklist from John Hopkins and Mc Master University to improve the delivery of the daily goals on ICU, including various sepsis-relevant questions. The results were presented in general fora in the respective departments after the initial audit and the re-audit.
Results: Quantitatively our performance data for ICU showed for the initial data collection 130 agreed and 110 documented goals (84.6% documentation). For the re-audit we found 180 agreed (+138%) and 174 documented (+158%) goals. Documentation was reached in the re-audit in 96.7% (including the sepsis relevant goals).
81.5% of the aims were achieved in the original audit and 86,1% in the re-audit. Reasons, that goals were not achieved laid initially in 79.2% inside our ICU and 16.7% outside (4.1% lacking data). For the re-audit we found 60% in our ICU and 40% outside. Reasons in ICU were divided into patient factors, organization and resource factors (initially: 16.7/54.2/8.3%; re-audit: 36/20/4%).
Qualitatively we identified a variety of reasons for the failure to achieve our goals. No reagents for basic, sepsis relevant investigations and therapies like the malaria rapid diagnostic test or electrolytes. The availability for the signatory allowed to sign for a MRI to determine a focus. The absence of blood in the blood bank or the absence of fever reducing medication. Another focus for the non- achievement of goals was lack of experience and expertise in the clinical setting and in interdisciplinary communication.
Conclusions: In order to reduce poor documentation, the failure to achieve our goals (especially in septic patients) and to reduce the length of hospital stay we suggested the introduction of a checklist, integrated meetings of all involved professional groups and regular re-audits.

021
Infection 2017

Impact of endogenous IgGAM-levels on outcome in sepsis—recent findings

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Introduction: The main function of immunoglobulins (Ig) during the immune response in sepsis is the clearance of pathogens, endotoxins, DAMPs and PAMPs by activation/regulation of the complement cascade [1]. It is known that in some patients the immunological response to sepsis is impaired and increased Ig production does not occur. This might be due to apoptotic depletion of memory B-cells [2, 3]. The prognosis of patients with sepsis depends on several factors such as load and virulence of the pathogen, effective antimicrobial therapy, and obviously also on age-dependant inefficiency of Ig

production [4]. In this context, IgM plays a major protective role, and in patients with decreased IgM levels mortality is extremely high [5]. However, whether adjunct treatment with exogenous IgM-enriched preparations improves survival in these patients is still under investigation and a matter of controversy [6].
Objectives: To summarize the available data on the effect of endogenous IgGAM levels on the clinical outcome of patients with severe sepsis or septic shock and to present data on the effect of adjunct therapy with IgM in such patients. This information may help to identify target groups for adjunct treatment of sepsis with intravenous IgGAM.
Methods: We searched PubMed entries up to June 2017 and summarized the relevant literature in a narrative review.
Results: We found 12 studies on this topic. In general, the potential effects of supplementation with Ig preparations depend on several factors, including severity of sepsis and efficacy of general therapy per se. With regard to IgM therapy, Ig levels should be assessed directly after admission of patients with severe sepsis or septic shock to the ICU. In such patients, decreased IgM concentrations may predict a higher mortality [7]. Consequently, these patients are a target group for treatment with exogenous IgM preparations. If a decision to supplement IgM is made, the time between diagnosis and IgM administration is a significant predictor of mortality risk, and treatment within <23 h after diagnosis is most beneficial to reduce mortality. The duration of therapy should be at least 72 h to achieve a sustained effect [6, 8]. However, before this therapy is initiated, the general prognosis has to be taken into account and this therapy should not be given to a patient whose condition makes it futile. In summary, the response of patients to treatment with IgGAM can probably be predicted on the basis of the above criteria.
Conclusions: Sepsis and septic shock are serious diseases with a high mortality even if standard therapy according to recent guidelines is applied. In patients with an impaired immune response, as detected by decreased levels of endogenous immunoglobulins (i.e. IgM levels), early adjunct therapy with an IgM-containing solution may improve survival. However, an early start and sustained application for 72 h seem mandatory.
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028
Infection 2017

Number needed to treat and cost per life saved of adjunctive igm-enriched immunoglobulin treatment of sepsis in Germany

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Introduction: Sepsis is a life-threatening clinical syndrome defined by a systemic inflammatory response to infection. In Germany, the expected number of newly diagnosed cases with severe sepsis amounts to 76–110 per 100,000 adult inhabitants. Despite the development of new antibiotics, mortality of patients with severe sepsis or septic shock in German ICUs remains high with about 34.4–55.2%. Adjunctive treatment with IgM-enriched immunoglobulins is one of the sepsis management strategies according to German guidelines.
Objectives: This research aims to investigate the number needed to treat (NNT) of IgM-enriched immunoglobulins in adult sepsis and compare it with other, widespread treatment strategies. The NNT will additionally be used to calculate the cost per life saved.
Methods: A number needed to treat model was developed by obtaining effectiveness data from a systematic literature review including the latest meta-analysis and subsequent trials, when available. The main outcome was mortality. Additionally, a cost per life saved model was developed for Germany using the list price of IgM-enriched immunoglobulins according to the public price list in Germany (Lauertaxe).
Results: Based on the latest Cochrane meta-analysis, the NNT of IgM-enriched immunoglobulin treatment in sepsis is 8. This was compared with a number of other treatments and their NNTs: rapid defibrillation for cardiac arrest (2,5), prophylactic antibiotics for reducing ICU respiratory tract infections (18), tranexamic acid for severe trauma (67), statins for heart disease prevention (83) and blood pressure medicines for hypertension (125). However, sepsis treatment developed during the last 10 years and when taking only the most recent studies including real-world data into account, the NNT for IgM-enriched immunoglobulins for adult sepsis patients is reduced to 3,93. The average treatment costs in Germany are 4106€. Therefore, the cost per life saved ranges between 16114€ and 32844€.
Conclusions: The findings suggest that the treatment of septic patients with IgM-enriched immunoglobulins is effective in reducing mortality compared to other therapies. Considering international cost-effectiveness thresholds, Pentaglobin represents a cost-effective use of health care resources. Therefore, IGM-enriched immunoglobulins can be recommended as adjunctive treatment of patients with sepsis.
Acknowledgements: This analysis was sponsored by Biotest AG, Dreieich, Germany.

031
Infection 2017

Clinical and microbiological characteristics of *Staphylococcus aureus* bacteremia in the Military University Hospital Prague

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Introduction: *Staphylococcus aureus* bacteremia (SAB) is the most common bloodstream infection with incidence of 15–40 cases per 100,000 population. Mortality of SAB caused by methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus* ranges between 20–40%. Previous studies documented the importance of infectious diseases (ID) expert consultations in the management of SAB and compliance to evidence-based guidelines in diagnosis and therapy.
Objectives: The aim of this study was to characterize a cohort of patients with SAB hospitalized during a two year period and evaluate the quality of SAB management in a tertiary care hospital.
Methods: A total of 65 patients with SAB hospitalized at all departments of the Military University Hospital Prague in 2015–2016 were retrospectively evaluated based on positive results of blood culture testing. Clinical and laboratory parameters were analysed and compliance of SAB management to current guidelines was evaluated.
Results: The mean age of patients was 65 years (range 20–94 years), with 42 men and 23 women with the mean hospital stay of 23 days (median 16 days). In 60 patients (92%), MSSA was detected and 5 cases of SAB (8%) were caused by MRSA. The mortality of 28% was documented in the study cohort, 23 and 80% in the MSSA and MRSA patients, respectively. Source of infection was found in 83% of SAB cases with catheter-related infections being the most common cause in 20% of patients followed by SAB due to surgery-related infections, skin and soft tissue infections, lung infections (all 14%), infective endocarditis (12%), bone infections (5%) and port infections (5%). The most common empirical antibiotic therapy included aminopenicillins and third generation cephalosporins. Upon SAB detection, only 45% of patients with MSSA etiology were switched to appropriate targeted antibiotic therapy. However, all patients with SAB due to MRSA received adequate antibiotics. Follow up blood cultures were properly collected only in 42% of patients and ID consultations were performed only in 23 (35%) patients. Interestingly, mortality in a group of 23 patients who were consulted by an ID physician was only 17%.
Conclusions: The management of patient with SAB must include recommended diagnostic and therapeutical interventions. It is necessary to rapidly detect and possibly remove infectious focus, promptly administer effective antibiotic therapy based on microbiological results, obtain follow up blood cultures and indicate appropriate duration of SAB treatment. Our retrospective study document a lack of compliance to these rules in clinical practice and support the importance of ID consults to improve the SAB management.
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Acknowledgements: The study was supported by a project MO 1012, SVV 260369 and UNCE 204022.

036
Infection 2017

Therapeutic plasma exchange as rescue therapy in refractory septic shock

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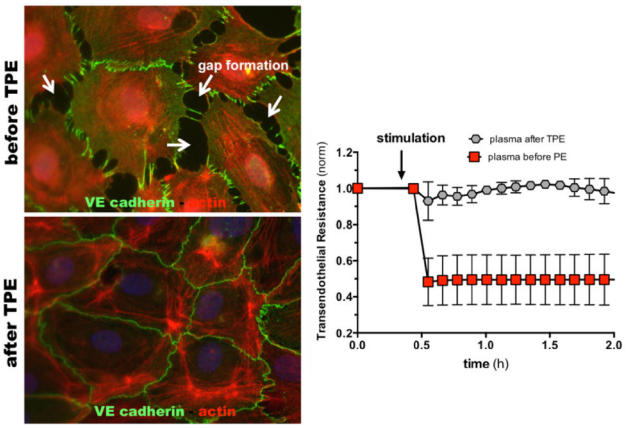
Introduction: Sepsis is a life-threatening dysregulated host response to infection. Given the injurious role of (1) the overwhelming immune response and (2) the consumption of protective plasmatic factors (e.g. vWF cleaving proteases—ADAMTS13 etc.) we hypothesize that early therapeutic plasma exchange (TPE) in severely ill individuals might be beneficial. TPE combines two aspects in one procedure: (1) removal of harmful circulating molecules and (2) replacement of protective plasma proteins.
Objectives: To investigate surrogate endpoints and feasibility of an add-on TPE strategy in early septic shock patients.
Methods: We have included 17 septic shock patients (onset <12 h) requiring high doses of noradrenaline (>0.4 g/kg/min). TPE was performed within 4 h. Clinical and chemical data were obtained longitudinally besides the evaluation of 28-day mortality. Plasma samples before and after TPE were obtained for stimulation of human umbilical vein endothelial cells (HUVECs) ex vivo to analyze their phenotype with regard to permeability (fluorescent immunocytochemistry and transendothelial electrical resistance (TER)).
Results: Mean ADAMTS13 activity was 36 ± 18%. The 28-day mortality in this study was found 24.3% lower (64.7%) as the predicted mortality (88.95%) by APACHE II score (37.6 ± 4). TPE resulted in hemodynamic stabilization as indicated by mean arterial pressure (63 ± 11 vs. 71 ± 9 mmHg, $p = 0.002$) and lower vasopressor requirement (NA 0.84 ± 0.4 vs. 0.55 ± 0.2 ug/kg/min, $p = 0.0006$). Fluid balance was also positively affected probably by reduced capillary leakage. This is supported by ex vivo stimulation of HUVECs with septic plasma where plasma before TPE induced severe alteration of cellular architecture including a disassembly of adherens junction (VE-cadherin IF) and a dramatic increase in permeability (TER, Figure). The same patients' plasma after TPE did not induce this typical septic phenotype.
Conclusions: This pilot study supports our hypothesis that early TPE in highly unstable patients might be beneficial with regard to hemodynamic stability, microcirculatory perfusion and overall outcome. A national multicenter randomized trial powered for mortality is highly desirable. Funding applications are currently under review.



049
Infection 2017

A mirror: sepsis management audit in emergency department (step 1.)

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Introduction: Besides the growing patient flow, the timely recognition and adequate urgent care of septic patients, who do not always show specific symptoms, is one of the greatest challenges for emergency care staff. In addition to this challenge there is the fact that the incidence is rising[(1)] and the recognition could be difficult.
Objectives: We investigated what kind of indicators could characterize the management of sepsis in the Emergency Department (ED) of Central Hungary with the highest patient flow.
Methods: A sepsis care audit was performed in the ED of our adult care hospital. We examined the first 1000 patient who entered our ED from 1st January 2017. The data of septic patients was separated from the total cases. Sepsis was defined by verified infection plus SOFA score $\geq$2. We investigated the incidences, the basic demographic data, the achievement of the Sepsis Six and the crucial time data as treatment indicators.
Result: The 1000 patients have been admitted within seven and a half days. In 163 cases acute infection was verified and in 63 cases sepsis was diagnosed (Figure 1). 46% of septic patients were women, 54% were men. The median age was 74 years, 82.5% of patients were over 60 years old. 14% of patients required vasoactive circulatory support and there was need for intensive care in 19% of the cases. Two patients died in the ED. During primary emergency care, Sepsis Six elements were totally ignored in 9 cases, while in 4 cases all requirements were completely fulfilled (Figure 2a and 2b), however, meeting all criteria did not show correlation with the SOFA score. Total of 25 patients (40%) received antimicrobial treatment in the ED, 75% in the intensive care subgroup. The median wait time from admission to medical examination was 25.5 min, which showed inverse correlation with the SOFA score describing the actual condition. The median time to the initiation of antimicrobial treatment (in those cases when it was administered) was 248 and 480 min in the intensive care subgroup (Figure 3). Septic patients had 580 min-long median turnaround time in the ED that showed linear relationship with the SOFA score (Figure 4). Those patients who needed to wait for admission to the ICU spent the longest time in the ED.
Conclusion: While epidemiological and demographic data seem to be similar to international data[(2)], there is a significant lag in emergency health care indicators compared to standards[(3)]. The short wait time of patients with need of intensive care, with their late antimicrobial therapy reflects the deficiency in conceptual thinking, while the sporadicity of the fully implemented Sepsis Six package and its independence from the SOFA score demonstrates the lack of knowledge of treatment standards. There is a need for system intervention designed to improve conceptual thinking relating sepsis, early recognition and standardized, provider-independent care.
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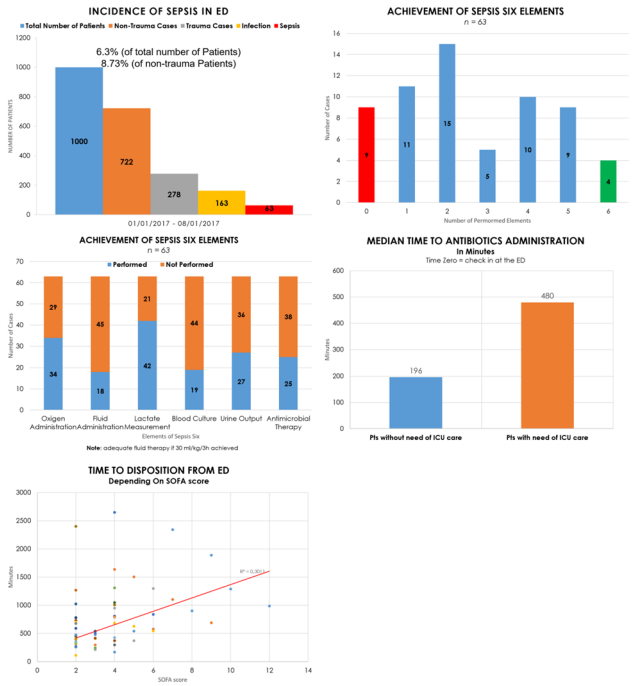

050
Infection 2017

"Sepsis Project": Complex Intervention Package to Improve Sepsis Management in Emergency Department (Step 2.)

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Introduction: Sepsis is an emergency condition in which the delay in treatment worsens outcomes[(1–3)]. According to the international guideline, antimicrobial therapy should be initiated within 1 h after recognition of sepsis[(4)]. This goal presents a challenge to the loaded Emergency Departments.
Objectives: As part of a complex program called Sepsis Project, we audited the sepsis care in the ED with the highest patient flow of Central Hungary. Based on the results, we started to implement an intervention package with the purpose to facilitate the treatment of sepsis according to international standards.
Methods: *1. Education.* A mandatory training day organized for ED's healthcare providers (doctors and nurses), consisted of three main elements: theoretical lecture, micro-skill practice and situation practice (Table 1). The purpose of the education was to introduce the Sepsis-3 definition, to promote early recognition, to present the

elements of the treatment, to describe the 2016 international guideline, thereby to improve the treatment following standards, to increase the survival rate of septic patients (Figure 1.). 2. Sepsis screening tool. We started to use a special document in the ED. The purpose of this tool is to facilitate the recognition of septic patients according to PIRO principles during the triage process and to lead the real-time management of the septic patients based on decision support (Figure 2a and 2b). 3. Re-audit. We plan to justify the improvement in the quality of treatment arising from the implementation of the intervention package by comparing the results of the re-audit with the previous one. *4. Organized cooperation.* This program in our department is not unique, the whole project is part of a program launched by the Hungarian Emergency Sepsis Network, set up at the beginning of 2017, which aims to assess and improve the quality of the primary care of septic patients in the Hungarian ED-s.

Result. 24 residents and specialists and 36 professional workers have participated in the education. The implementation of the complex intervention package is ongoing and the training of the entire staff requires additional occasions. We are looking forward to see improvement in the recognition at triage, in the achievement of Sepsis Six and in the timing of the antimicrobial treatment initiation. The objective justification for these measurable changes is the planned task of a subsequent re-audit ("Step 3"). The Hungarian Emergency Sepsis Network has begun with the collaboration of 5 Hungarian ED-s. Since then more than 20 hospitals have joined the program from several parts of the country.

Conclusion: Comprehensive interactive education organized for emergency care staff and the use of a tool during treatment to support recognition and therapeutic decision are expected to improve the quality of septic patient's care.

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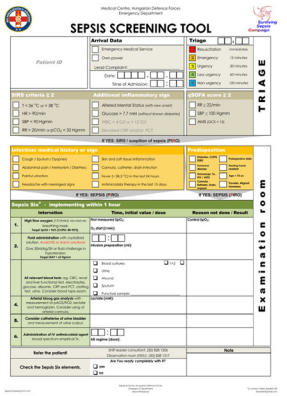

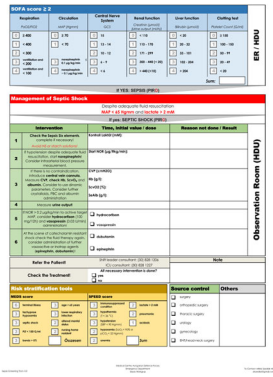

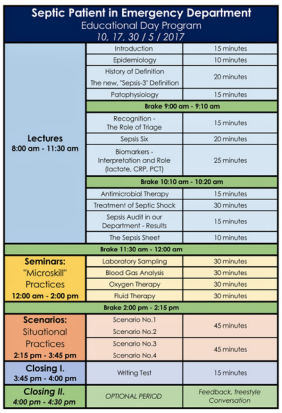

053
Infection 2017

Succesful treatment with Cytosorb® in a case of septic shock, ARDS, multiorgan failure and purpura fulminans due to Acinetobacter baumannii pneumonia

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Introduction: In several studies and in in-vitro data is demonstrated that treatment with an extracorporal cytokine adsorber (CytoSorb®)

may be useful in patients with septic multiple organ failure or other critical diseases due to an excess of cytokines. This therapy has meanwhile been used in over 500 hospitals worldwide, more than 23000 applications, is well tolerated and safe. Purpura fulminans is a rare disease with thrombosis and tissue necrosis caused by protein C deficiency. Data on this life threatening disease are rare, treatment guidelines have not been established.

Objectives: We treated a patient with septic shock, ARDS, multiorgan failure and purpura fulminans due to community acquired Acinetobacter baumannii pneumonia with ECMO, protein C and Cytosorb®.

The Patient without any chronic diseases beside alcohol abuse (male, 47 years old) has been admitted to our ward from another hospital, ventilated and in septic shock. At admission APACHE II 38, heart rate 133 bpm, MAP 60/Norepinephrine 2.8 mg/h, fever 38.5 °C, severe ARDS (paO2/fiO2 95).

Methods: Initial protocol treatment (lung protective ventilation, volume resuscitation) failed; we treated therefore with ECMO (X-Lung®, Novalung), CVVHD (Fresenius multifiltrate) and as adjunctive therapy with Cytosorb® haemadsorption (7 applications). In initial treatment we changed the adsorber every 12 h, from day 3 we changed every 24 h. Adsorber was used with CVVHD (Citrat anticoagulation) in pre-filter position, blood flow 150 ml/min. Protein C 3000 IE was applicated.

Results: ECMO could be weaned after 10 days (paO2/fiO2 308), after 7 cycles of Cytosorb therapy we reached shock reversal (Norepinephrine 0.5 mg/h, MAP 85) and catecholamines could be terminated several days later. After application of 3000 IE protein C tissue necrosis stopped immediately and plasma protein c levels normalized (from 26% up to 83%). SOFA decreased from 15 to 10, SAPS 2 from 58 to 43, other laboratory and physiological data are shown in table 1. After 10 days we started weaning from ventilator, patient was awake and vigilant, but ventilation had to be continued because of severe Critical ill PNP and tetraparesis. In the next 4 weeks patient developed stable clinical conditions. Catecholamine demand even as intermittent dialysis could be finished, skin necrosis went better, no surgical intervention was necessary of it. Only ventilation had to be continued because of the persistent tetraparesis caused by CIP. After 57 days of ICU treatment we transferred our patient in good clinical conditions to a rehabilitation Center.

Conclusions: We reached complete shock reversal after 7 applications of Cytosorb therapy even as complete restitution of the purpura. Treatment with CytoSorb® adsorber in a patient with sepsis acquired purpura fulminans had shown great effect, been safe and without any side effects

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Hemoadsorption in infection-associated rhabdomyolysis. Suefke S et al, Ther Apher Dial. 2016

Day	Protein C (%)	NOR/ MAP (µg/h/mmHg)	PCT (ng/ml)	CRP (mg/L)	Krea (mg/dl)	CK U/L	Lactate (mg/dl)	WBC *10³/µl	paO2/fiO2 mmHg
admission	26%	40	203	471,7	1,64	317	17	1,41	97
1		20	245	667,8	2,66		27	4,53	126
2		18,8	185	504,3	2,52	1459	32	16,64	108
3	83%	4,7	139	341,5	2,09	1851	25	25,86	117
7		7,1	87,8	87,8	1,99	307	10	26,48	210
14		3	6,14		5,43	1591	5	23,22	228
28		0		15,21	3,11		10	17,39	323
transfer		0	0,2	54,8	0,37	13	10	14,6	437

064
Infection 2017

Assessment of some factors associated with death in patients from necrotizing fasciitis

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Introduction: Necrotizing fasciitis (NF) is life-threatening disease among surgical infections. It characterized as rapid necrosis of fascia, soft tissues, sepsis, septic shock and still high mortality rate. The prognostic factors associated with death from NF need to be analyzed.

Objectives: To assess some factors associated with death from NF.

Methods: The medical cases of 55 patients with NF who admitted to the department of surgical infections and sepsis were analyzed retrospectively. Demographic, Co-morbidity, clinical data and laboratory tests were compared between patients who survived and did not.

Results: 55 patients with NF divided into Group 1 (survived)—54.5% and Group 2 (died from NF)—45.5%. Two groups had no differences between gender data and wound location ($p = 0.068$). Group 2 characterized with greater amount of Septic shock cases 68.4 and 31.6% in Group 1 ($p = 0.074$). Patients in group 2 had average age 70.6 and in group 1 50.4 ($p = 0.049$). Time from disease's onset and admitted to hospital in group 1 was about 7.9 h and in Group 2–72.8 h ($p = 0.045$). Patients in Group 1 had average 3,4 debridement compared with 1.9 in group 2 ($p = 0.049$). Average length of stay in ICU was 14.2 days in group 1 which was significantly greater ($p = 0.035$) than patients in group 2 who spent 7.3 days. SOFA scores were 6.3 in Group 1 and 15.8 in Group 2 ($p = 0.048$). Laboratory values were similar in both groups (Hb, WBC, PLT, MPV, PWD, creatinine, total bilirubin, glucose level, Alt) and showed no statistically significant differences between groups ($p = 0.089$).

Conclusions: In this study patients who died from NF were older age group, late admitted to the hospital. Also this group characterized with majority of septic shock and statistically significant SOFA scores. The earliest disease's onset, aggressive surgical treatment (Debridement) showed better outcome in Group 1. We found no correlations between prolonged stay in ICU and risk of death.

065
Infection 2017

Effects of time to source control on 28-day-mortality in patients with severe sepsis or septic shock

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Introduction: Guidelines recommend timely source control in patients with (severe) sepsis and/or septic shock. Yet, only in the recent SSC guideline source control is recommended "as soon as possible" [1]. The former SSC- as well as the German S2k guidelines recommended source control within 12 or 6 h, respectively, yet

without indicating any underlying data or level of evidence [2, 3]. The current SSC recommendation is based on results of the observational phase of our MEDUSA trial, showing a significant effect of timely and successful source control on mortality [4].
Objectives: To investigate the effects of time to source control and success of source control on survival in patients with severe sepsis or septic shock.
Methods: Secondary analysis of the MEDUSA study [4], a cluster randomized controlled trial including patients with severe sepsis or septic shock treated on the ICU. Time-to-source control and success of source control were abstracted from patient records by intensive care clinicians. We analyzed the effect on 28-day-mortality over both intervention phases of the MEDUSA trial [5]. Analyses: Uni- and multivariate generalized hierarchical linear models controlling for clustering of data.
Results: 2496 of a total of 6576 patients received surgical focus sanitation. Delay of source control of more than 6 h was observed in 767 (31%) patients, source control was unsuccessful in 351 (14%) patients. Mortality increased with delay (≤6h: 28%, >6h: 38%) and non-success of source control (success: 25%, non-success: 65%). Controlled for risk-factors, delay of source control had an effect on source control being unsuccessful (OR = 1.65 [95% CI 1.24–2.18], $p \leq 0.001$). Both delay of source control and unsuccessful source control had an effect on survival (OR = 1.38 [1.09–1.75] and OR = 6.23[4.55–8.55], respectively, both $p \leq 0.007$). Time to source control had an effect on 28-day-mortality both among patients where source control was judged to be successful and patients where it was judged to be unsuccessful (OR = 1.31 and OR = 1.76 respectively, both $p \leq 0.045$).
Conclusions: Time to source control in patients with severe sepsis or septic shock has significant effects on success of source control and survival. Therefore, capacities for diagnosis and intervention should be provided at all times in any institution caring for patients at risk of sepsis with a surgical focus.
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Acknowledgements: The study was funded by the German Federal Ministry of Education and Research via the integrated research and treatment center "Center for Sepsis Control and Care" (FKZ 01EO1002).

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Effects of a multimodal intervention using change-management methods on guideline adherence and 28-day mortality: results of a prospective controlled multicenter study

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Introduction: Early antimicrobial treatment (AT) is an essential part of guidelines on treatment of sepsis [1]. Several quality improvement (QI) initiatives were able to increase guideline adherence and to reduce sepsis-related mortality but used uncontrolled before-after designs [2].
Objectives: To investigate the effects of applying change-management methods to improve the quality of antimicrobial therapy and decrease sepsis-related mortality in a prospective controlled trial.
Methods: Secondary analysis of the MEDUSA study [3], a cluster randomized controlled trial including patients with severe sepsis or septic shock treated on the ICU. Hospitals were randomized into an intervention and a control group in study phase 1. After two years, hospitals switched groups for study phase 2. Intervention: Audit and feedback of quality indicators, change-management methods (local QI teams, change-facilitation by study team); Control in phase 1: Standard medical education; in phase 2: Feedback. Analyses: Differences between study phase and surveillance phase regarding study outcomes were compared between groups (difference-in-differences [d-i-d] design). Generalized hierarchical linear models controlling for clustering of data; Test of d-i-d by interaction effects between study phase and study group.
Results: 40 hospitals participated in the trial including 4182 patients in the intervention phase and 2394 patients in the surveillance phase. Baseline characteristics showed substantial differences between groups because of randomization failure (median SAPS-II group 1: 50, group 2: 46). Percentage of patients receiving antimicrobial therapy within 1 h did not change in group 1 and increased in group 2 (38 vs. 40%, clustering-adjusted OR [95% CI] = 0.96[0.8–1.1], and 34 vs. 42%, OR = 1.3[1.1–1.6] respectively, d-i-d: $p = 0.016$). Percentage of patients with de-escalation of AT within 5 days showed a similar development (OR = 1.1[0.9–1.3] and OR = 1.6[1.3–2], d-i-d: $p = 0.012$). Percentage of at least 2 sets of blood cultures taken increased in both groups (OR = 1.4[1.2-1.7] and OR = 1.7[1.4-2], d-i-d: $p = 0.138$). There was no d-i-d in 28-day mortality (35 vs. 34%, OR = 0.95 [0.8–1.1] and 27 vs. 28%, OR = 1.1 [0.9–1.3], d-i-d: $p = 0.313$), which stayed true when controlling for risk-factors (d-i-d: $p = 0.636$).
Conclusions: This is the first controlled trial showing an effect of QI interventions on time to first antimicrobial therapy. However, these changes in guideline compliance were obviously not marked enough to result in improved patient survival.
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Infection 2017

SMARTDOSE—Simultaneous determination of multiple antibiotics by LC-MS/MS in plasma, tissue, and breath condensate for personalized antibiotic treatment

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Introduction: Adequate antibiotic treatment is the prerequisite for successful treatment of systemic infections. Increased scientific evidence shows that a fixed dosage regimen can lead to insufficient and ineffective antibiotic therapy. Thus, personalized therapy regimes by Therapeutic Drug Monitoring are necessary.
Objectives: The aim of this study was to develop and validate a reproducible and robust method for quantification of antimicrobials by using liquid chromatography with mass spectrometric detection (LC-MS/MS) in different biological specimens (plasma, tissue, exhaled breath condensate).
Methods: The method was developed for simultaneous quantification of nine antimicrobials (Aciclovir, Ampicillin, Cefuroxime, Ciprofloxacin, Meropenem, Metronidazole, Piperacillin, Rifampicin, Tazobactam) in lithium-heparin plasma, entailed a single method for sample preparation, enabling quick processing of the samples followed by an LC-MS method with a chromatographic run time of 10 min. The method was validated for sensitivity, specificity, linearity, accuracy, precision, dilution integrity according to the guidelines for bioanalytical method validation of the European Medicines Agency. This new method was also applied for determination of antimicrobial concentration in other materials, e.g. tissue, exhaled breath.
Results: All calibration curves were linear over a range of 1–100 mg/l, and if necessary, samples can be diluted 2- or 5-fold. Furthermore, the accuracy was between 85.1 and 114.9% for all analytes, and the within- and between-run precision were <16.5% for the lower limit of quantification and <14.8% for the middle level and upper limit of quantification. Preliminary data show a possible application of the newly established quantification method also for other materials than plasma, e.g. tissue, exhaled breath. Further validation for this other materials is in preparation.
Conclusions: A short analysis time, small amount of plasma needed, high specificity, and accuracy make the LC-MS/MS method developed in this study appropriate and practical for therapeutic drug monitoring of antimicrobials in routine analysis.

075
Infection 2017

Glu-plasminogen—a life-saving drug for patients with organ failure?

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Introduction: A functioning fibrinolytic system is the prerequisite for the patency of blood vessels. For this, the balance between the central protein of the fibrinolytic system Plasminogen (Plg) and the main Plasmin Inhibitor Alpha2-Antiplasmin (APL) has to be warranted. Under certain circumstances (e.g. severe septicaemia), however, the APL level of the patients plasma exceeds the Plg level markedly, leading to persistent clots in the capillaries and thus to organ failure (e.g. the kidney) and finally in severe cases to the death of the patients. In Germany die annually up to 70,000 patients due to these problems. Those are about 50% of the affected persons.
Objectives: Since the components of the fibrinolytic system—Plg and APL—are normally not considered in the diagnosis and treatment of those patients, we tried to see if the substitution of plasma components including Glu-Plasminogen could improve the survival of those patients, and also looked for the effect of the substitution of Glu-Plasminogen in patients with acute renal failure.
Methods: Citrated plasma was used for the determination of the proteins Plg, APL and ATIII using the respective Berichrome testkits of Behringwerke, Marburg. Glu-Plasminogen was obtained by affinity chromatography from Cohn I fraction supernatant of plasma fractionation.
Results: In a small open clinical study, 14 patients with severe septicaemia and septic shock, were treated additionally to the conventional treatment with plasma components including Glu-Plasminogen. The control group—19 patients—obtained only the conventional treatment for septic patients. In the control group 9 patients died (47%), whereas in the group which was additionally treated with plasma proteins including Glu-Plasminogen only 3 patients died (21%).
A further study including patients with acute renal failure showed also very positive effects of the substitution of Glu-Plasminogen. Kidneys opened in close time relation to the substitution of Plg and the creatinine clearance normalysed.
Conclusions: Our results show the very positive effect of the substitution of Glu-Plasminogen to balance the higher level of APL in patients with multi organ failure. The acute death rate of these patients could be reduced by more than 50%. If these results could be verified in a double blind clinical trial, this approach to treat patients with multi organ failure using plasma proteins including Glu-Plasminogen would save about 30,000 patients annually alone in Germany from acute death. Thus, this approach would be the most promising method to save the lives of those patients.

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The influence of comorbidities on mortality in medical intensive care patients with severe sepsis and septic shock

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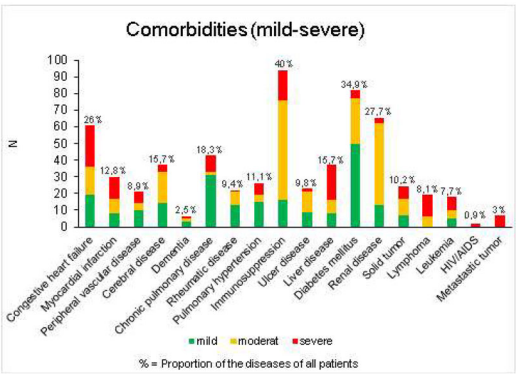
Introduction: Despite all improvements in sepsis care hospital mortality of patients with severe sepsis and septic shock is still in the range of 30–50%. It is known that certain comorbidities predispose to sepsis and are associated with a high mortality.
Objectives: The aim of the study was to investigate the relevance of comorbidities as a contributing or leading cause of death in sepsis patients.
Methods: Retrospective analysis of 235 patients with severe sepsis/septic shock, age >18 years and no treatment restrictions admitted to the Medical ICU of the University Hospital Tübingen, Germany, from 12/2010 to 05/2015. Comorbidities were assessed by the Charlson Comorbidity Score (CCS), which was also modified to assess different severities of comorbidities (Charlson Score of the Severity of Comorbidities, CSSC). In addition, SAPS 2, SOFA, Sepsis Severity Score (SSS) and KNAUS score was determined for all patients. Finally, 4 different raters (PhD student, medical intensive care attending, anesthesiologist and medical specialists for the dominating comorbidity) reviewed the medical documentation of each non-survivor to subjectively grade the contribution of the comorbidities to mortality into 4 categories.
Results: Hospital mortality was 33.2%(78/235). Mean SOFA score was 11.0 (CI 10.1–11.8) in non-survivors and 8.4 (CI 7.9–9.0) in survivors. Standardized mortality ratio was 0.73 based on the SAPS 2 and 0.65 based on the SSS. Frequency and severity of comorbidities are shown in Fig. 1. Mortality rate ranged from 15.6% in patients with low CSSC (0–1, n = 24) to 56.3% in patients with very high CSSC

(≥10, n = 22). Mean CSSC among the non-survivors was 5.7 (CI 5.0–6.4) and in survivors 4.8 (CI 4.3–5.2). Age <50 years was associated with a lower mortality (21.6%). An immunosuppression had 40% of all patients and 50% of non-survivors. Mortality was highest in patients with severe chronic pulmonary disease/pulmonary hypertension (52.9%, n = 9/17), severe malignant diseases (48.6%, n = 17/35) and severe liver disease (47.6%, n = 10/21). Only 5.1% of non-survivors had a normal health status (KNAUS A) before hospital admission, whereas 41.0% were bedridden (KNAUS D). Sepsis was subjectively rated as solely responsible for death on average in 7.7%, sepsis as the leading cause of death with relevant comorbidities present in 31.4%, sepsis and relevant comorbidities equally contributing in 32.7% and comorbidities as the leading cause of death in 28.2% of fatal cases. Gradings of the 4 individual raters are shown in Tab. 1.

Conclusions: Significant comorbidities were very common in our cohort of medical ICU patients with severe sepsis and septic shock and were subjectively perceived as a major contributor or the leading cause of death in the majority of patients.

Reason for death of patients with severe sepsis	Non-survivors	CSSC	Sepsis Severity Score	SOFA
	N (%)	mean (SD)	mean (SD)	mean (SD)
Assessment reason for death (Medical doctoral student)				
Cause of death sepsis	9 (11,5)	1,3 (1,4)	87,2 (17,7)	11,2 (4,2)
Sepsis is the leading cause of death and relevant comorbidities	19 (24,4)	4,8 (1,8)	86,1 (22,3)	11,3 (4,5)
Sepsis and comorbidities are cause of death	35 (44,9)	7,1 (2,8)	83,8 (17,6)	10,7 (3,2)
Comorbidities are the leading cause of death and sepsis only accompanying	15 (19,2)	6,3 (2,9)	74,6 (18,9)	11,1 (3,5)
Assessment reason for death (Medical ICU specialist)				
Cause of death sepsis	5 (6,4)	1,2 (1,3)	91,6 (20,0)	12,6 (5,3)
Sepsis is the leading cause of death and relevant comorbidities	12 (15,4)	4,9 (2,7)	86,4 (18,4)	10,9 (4,7)
Sepsis and comorbidities are cause of death	33 (42,3)	6,1 (2,7)	82,2 (20,0)	10,7 (3,5)
Comorbidities are the leading cause of death and sepsis only accompanying	28 (35,9)	6,3 (3,1)	79,5 (19,9)	10,8 (3,6)
Assessment reason for death (Medical specialist)				
Cause of death sepsis	5 (6,4)	1,6 (1,5)	75,2 (6,5)	10,4 (4,4)
Sepsis is the leading cause of death and relevant comorbidities	26 (33,3)	5,6 (3,0)	85 (16,7)	11 (3,6)
Sepsis and comorbidities are cause of death	16 (20,5)	5,7 (2,0)	85 (22,7)	10,7 (3,5)
Comorbidities are the leading cause of death and sepsis only accompanying	31 (39,7)	6,4 (3,2)	80,3 (20,8)	11 (3,9)
Assessment reason for death (Anesthetist)				
Cause of death sepsis	5 (6,4)	2,2 (2,0)	85 (18,8)	10,4 (4,3)
Sepsis is the leading cause of death and relevant comorbidities	41 (52,6)	5,4 (2,8)	83,3 (19,2)	10,9 (3,7)
Sepsis and comorbidities are cause of death	18 (23,1)	6,6 (2,9)	83 (24,0)	10,7 (4,0)
Comorbidities are the leading cause of death and sepsis only accompanying	14 (17,9)	6,8 (3,3)	81,1 (13,4)	11,8 (3,2)

SOFA= Sequential Organ Failure Assessment Score; CSSC= Charlson Score of the Severity of Comorbidities



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Comparison of procalcitonin and C-reactive protein levels and blood culture results in septic patients

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Introduction: Procalcitonin (PCT) and C-reactive protein (CRP) are the most commonly used biomarkers in the sepsis diagnosis. However, their reliability is limited and the results should always be interpreted in the context of specific clinical situation. Dynamic of individual parameters in time is more important than their absolute values.

Clinical benefit of PCT monitoring lies in its ability to serve as negative predictive marker of sepsis and bacteremia and can suggest success or failure of initiated therapy.

Objectives: The objective of this study was to compare blood culture at the time of clinical diagnosis of severe sepsis (T0) with the procalcitonin and C-reactive protein serum levels at the time T0 and after 24 h.

Methods: During the years 2013 to 2016 95 blood cultures were examined in ICU patients with diagnosis of severe sepsis. Simultaneously, samples were sent for laboratory evaluation of PCT and CRP serum levels. After 24 h the control levels of procalcitonin (ΔPCT) and C-reactive protein (ΔCRP) were examined.

Data were divided into 5 groups: BC− (negative blood culture), BC+ (positive blood culture)—bacteremia, BC+G+ (only gram-positive blood culture), BC+G− (only gram-negative blood culture) and BC+PM (polymicrobial positive blood culture).

Basic statistic methods were used for analysis of collected data. Non parametric Kruskal-Wallis and Wilcoxon tests were applied to assess difference between cases in time or between individual groups.

Results: Out of total 95 blood samples were 57(60%) in BC−, 38(40%) in BC+ of which 24(63%) BC+G+, 8(21%) BC+G− and 6(16%) BC+PM (not further evaluated in this work). Respective values of PCT, ΔPCT, CRP and ΔCRP are shown in Table 1.

In the BC-group was found a significant increase of CRP levels after 24 h. Concentration of PCT ≥ 2.0 ng/ml was found in 23(40%) and PCT ≥10 ng/ml in 10(18%) of all samples. PCT levels together with ΔPCT <0.2 ng/ml were found in 3(8%) samples.

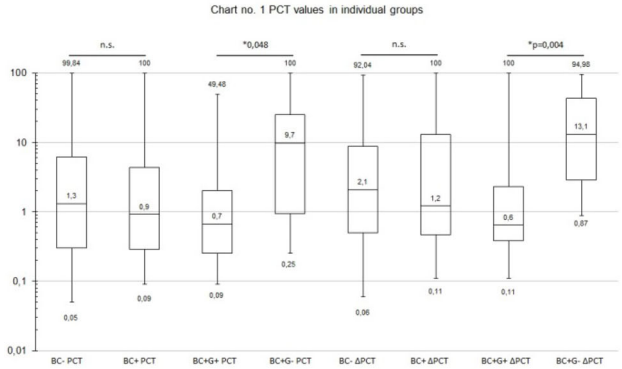
Comparison of the PCT and CRP level changes within the 24-h interval is shown in charts 1 and 2. Significant differences of PCT, CRP, ΔPCT and ΔCRP levels between BC+ and BC- groups were not observed. There was a significant difference in concentrations of PCT as well as ΔPCT between BC+G+ and BC+G-. Neither the CRP nor ΔCRP reached statistically significant values in the above mentioned subgroups.

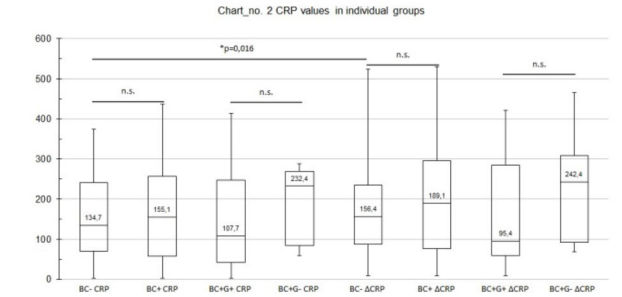
Conclusions: In our study we have failed to prove significant difference in PCT levels from bacteremic and non-bacteremic septic patients.

Significant difference in PCT and ΔPCT levels was noticed between gram-positive and gram-negative bacteremia groups with higher PCT a ΔPCT in gram negative bacteremia.

Negative predictive cut-off value of PCT for ruling out bacteremia based on our data was found to be PCT<0,2ng/ml

Acknowledgement: Supported by Ministry of Health, Czech Republic—conceptual development of research organization (Thomayer Hospital-TN, 00064190).





Group Median (Min. - Max.)	N - cases	PCT (ng/ml)	Δ PCT (ng/ml)	CRP (mg/l)	Δ CRP (mg/l)
BC-	57	1,3 (0,05-99,8)	2,1 (0,06-92,0)	134,7 (2,3-374,0)	156,4 (8,5-522,9)
BC+	38	0,9 (0,09-100,0)	1,2 (0,1-100,0)	155,1 (1,8-437,1)	189,1 (9,4-529,0)
BC+ G+	24	0,7 (0,09-49,5)	0,6 (0,1-100,0)	107,7 (1,8-413,0)	95,4 (9,4-422,9)
BC+ G-	8	9,7 (0,3-100,0)	13,1 (0,9-95,0)	232,4 (58,4-288,0)	242,4 (69,0-466,0)

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The role of enterococci in the treatment of patients with severe sepsis and septic shock

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Introduction: Enterococci belong to the most common pathogens involved in hospital-associated infections and frequently cause bloodstream infections[1]. Furthermore, enterococci are important pathogens in gram-positive sepsis and intraabdominal infection that may cause endocarditis and should be treated in multimorbid patients[2]. Whether a detection of enterococci from abdominal drainages or intraabdominal swabs (in absence of a positive blood culture) should be treated with antibiotics is still the subject of controversial discussions. Several studies failed to show a benefit of an empiric antibiotic therapy including enterococci species[3].
Objectives: The aim of the study was to evaluate whether enterocci effective antibiotics (Vancomycin, Teicoplanin, Tigecycline, Linezolid, Daptomycin and Aminoglycosides) may influence 90-day mortality in patients with intraabdominal enterococcal findings and severe sepsis or septic shock but without enterococcal positive blood cultures.
Methods: This retrospective monocentric observational study is based on data from the surgical intensive care unit of the University Hospital Greifswald from 2010 to 2013. The local ethics committee approved the study. Patients with severe sepsis and septic shock, who have received an abdominal swab during this period were included. Patients with positive detection of enterococci in blood cultures were excluded. The primary endpoint was 90-day mortality.
Patients were stratified into four groups according to the administration of enterococci effective antibiotics:
Gr. 1 (n = 70): pos. abdominal enterococci findings and administration of enterococci effective antibiotics
Gr. 2 (n = 55): pos. abd. enteroc. findings without administration of enterococci effective antibiotics .
Gr. 3 (n = 49): neg. abd. enteroc. findings and admin. of enterococci effective antibiotics
Gr. 4 (n = 91): neg. abd. enteroc. findings without admin. of enterococci effective antibiotics
Results: 265 patients were included (group 1 n = 70; group 2 n = 55, group 3 n = 49, group 4 n = 91). In all, 125 patients (47%) were tested positive of enterococci. 90-day mortality was reduced to 35% (25/70) in group 1 compared to 53% (29/55) in group 2 (OR: 2.01; 95% CI: 0.98–4.13; p-value: 0.06) but failed to demonstrate statistical significance.
53% (140/265) had no enterococci findings. In group 3 90-day mortality was 59% (29/49) compared to 40% (36/91) in group 4 (OR: 0.45; 95% CI: 0.22–0.92; p-value: 0.03).
Conclusions: In our small observational study the administration of enterococci effective antibiotics in septic patients with positive intraabdominal enterococci findings did not significantly reduce 90-day mortality.
Conversely, 90-day mortality was significantly increased in enterococci-negative septic patients that received enterococci effective antibiotics.
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Infection 2017

CytoSorb®—is anti-aging?

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Introduction: RAGE, the receptor for advanced glycation end products, binds to a broad repertoire of ligands including advanced glycation endproducts (AGEs)[1]. Soluble RAGE (sRAGE) may interfere with RAGE-ligand induced inflammation[2] such as increased expression of cytokines, chemokines, and adhesion molecules. Results of previous studies also emphasized the role of RAGE in murine models of sepsis by focussing on the anti-inflammatory role of IL-10[3].
Objectives: We here addressed changes of inflammatory biomarkers, sRAGE, AGEs in patients with septic shock before and after CytoSorb® treatment and asked for their correlation with immune phenotypes and clinical parameters i.e. recovery from septic shock and catecholamine dependency.
Methods: Inflammatory cytokines and soluble receptors were measured with validated ELISA techniques (Siemens Immulite1000®), sRAGE, AGE's, oxidized DNA(oxoDNA) were quantified by ELISA from MyBiosource Inc, CellCiolabs, CaymanChem. Leukocyte subpopulations were investigated by FCM (FACScalibur, BDBioSciences.com). Antibodies against CD antigens on T-, B-, and

myeloid cells were used to characterize activation states as well as immune inactivation.

Fiveteen patients were tested before and after CytoSorb® treatment. Two patients with an excellent clinical response and two patients with an inferior clinical improvement after Cytosorb® treatment were studied in greater detail.

Results: Before CytoSorb® treatment sRAGE concentrations ranged between 372 and 4486 pg/ml (median 1336pg/ml). CytoSorb® treatment resulted in reduced sRAGE levels in 2/4 patients (median 387 pg/ml). A rapid hemodynamic improvement within 24 h after CytoSorb® treatment was seen in 2/4 patients all these rapidly improving patients showed lowered sRAGE levels.

CytoSorb® treatment also resulted in decreased AGE concentrations in 3/4 patients (median before 2943 μg/ml to after 13.82 μg/ml). Another RAGE-ligand oxoDNA showed a clear reduction ($p = 0.14$) in 3/4 patients after CytoSorb® (median before 10,108 pg/ml to after 5210 pg/ml). Accordingly inflammatory cytokines such as IL-6 and sCD25(4/4); IL-8, IL-1βb and IL-10(3/4); were reduced after CytoSorb® treatment.

When comparing CD11b mean expression densities on granulocytes after CytoSorb® treatment we observed a significantly reduced expression in 3/4 patients. We also observed a decline of CD36 expression density on monocytes in 3/3 patients.

Conclusions: Plasma samples of CytoSorb® treated patients which were analyzed by our extended marker protocol verified that hemodynamic improvement was related to decreased IL-6, sCD25, IL-8, IL-1, IL-10 sRAGE, AGEs and oxoDNA. Leukocytes of patients who improved clinically had decreased expression densities of CD11b, and the scavenger receptor CD36 levels were found. The new observation of CytoSorb® to influence sRAGE and its receptors in patients recovering from septic shock deserves more detailed investigations.

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RAGE-mediated suppression of interleukin-10 results in enhanced mortality in a murine model of Acinetobacter baumannii sepsis.
Noto MJ et al

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Effect of an extended hemodynamic monitoring on therapeutic interventions and mortality in patients with sepsis—results from the VISEP-trial

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Introduction: Current guidelines recommend dynamic measures for guiding hemodynamic stabilization in sepsis patients [1]. In general, extended hemodynamic monitoring is only recommended in patients not responding to initial resuscitation [2]. However, data about the rational application of extended hemodynamic monitoring in sepsis patients are limited.

Objectives: To retrospectively analyze data from the VISEP-trial [3] regarding the association between application of extended hemodynamic monitoring as well as therapeutic interventions and mortality.

Methods: The VISEP trial was a randomized multicenter bifactorial trial involving 537 patients to investigate the effects of fluid resuscitation with Ringer's Lactate versus HES 10% and conventional versus intensive insulin therapy. The use of extended hemodynamic monitoring was not directed by the study protocol and was at the discretion of the treating physician. Patients were included into this analysis if hemodynamic monitoring with pulmonary artery catheter, transpulmonary thermodilution or absence of such a monitoring within 24 h after enrolment was documented.

Results: 526 patients—223 with (HEMO) and 303 patients without (NO-HEMO) extended hemodynamic monitoring—were included into this analysis. HEMO was associated with a higher APACHE-II score (21.6 ± 6.8 vs. 19.1 ± 6.2, $p < 0.001$) and a higher baseline serum lactate (3.9 ± 3.4 vs. 2.7 ± 3.5 mmol/l, $p < 0.001$). HEMO was also associated with more therapeutic interventions (Table 1). The 90-day mortality in the HEMO group was 45.3 versus 29.9% in the NOHEMO group ($p < 0.001$). When adding APACHE-II score and total volume of fluid resuscitation to a multivariate analysis, extended hemodynamic monitoring was independently associated with a higher 90-day mortality (OR = 1.5 [95% CI: 1.005–2.248]).

Conclusions: Patients receiving extended hemodynamic monitoring were sicker resulting in more aggressive fluid resuscitation and catecholamine therapy. Extended hemodynamic monitoring was independently associated with a higher mortality.

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Acknowledgements: The VISEP-trial was supported by a grant (01 KI 0106) from the German Federal Ministry of Education and Research and by unrestricted grants from B. Braun, HemoCue, and Novo Nordisk.

Table 1: therapeutic interventions (mean ± standard deviation)

	NO-HEMO	HEMO	p-value
Max. norepinephrine dosage [μg/kg/min]	0.27 ± 0.55	0.43 ± 0.54	<0.001
Max. dobutamine dosage [μg/kg/min]	4.9 ± 2.9	6.3 ± 3.9	0.004
Total Fluid resuscitation (Ringer's Lactate) [ml]	1793 ± 1648	2477 ± 2326	0.007
Total Fluid resuscitation (HES10%) [ml]	659 ± 382	748 ± 375	0.129
Renal replacement therapy	48/299 (16.1%)	83/223 (37.2%)	<0.001

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Use of Decasan antiseptic for local treatment of different forms of diabetic foot syndrome

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Introduction: The development of purulonecrotic complications (PNC) in diabetes mellitus can lead to amputation of lower limbs or

even death [1, 2]. Diabetic ulcers, infected with *Staphylococcus aureus* , increase the risk of death in 5 years [3]. As a result of aggression and low sensitivity, the infected lesions of skin in diabetic foot syndrome (DFS) tend to spread rapidly and require active surgical treatment, which often ends with amputation. That is why the role of local and general antimicrobial therapy cannot be overstated [4, 5].

Objectives: to define the composition of microflora in patients with different forms of DFS and the effect of chemical and biological antiseptics on them.

Methods: The exudate, washing of the ulcer surface and fragments of infected tissue served as materials for bacteriological study. Primary cultures were reviewed in 48 h and then every two days. The duration of cultivation with no growth was seven days. 210 bacteriological studies with identification of sensitivity to antibiotics were conducted.

Results: The infection in patients with PNC of DFS is characterized by its polyvalent nature caused by the presence of both aerobic and anaerobic microorganisms. The most frequent were aerobic bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*. The mixed infections, such as *S. aureus+Peptostreptococcus* spp., *S. aureus+Peptococcus* spp. and *S. epirdermidis+Enterobacteria+Bacteria fragilis* were also frequently observed. The frequency of anaerobic bacteria was 65.7% in patients with neuropathic form of DFS without pronounced inflammatory reaction and 94.5% with acute advanced inflammation. The similar tendencies were observed in patients with ischemic form of DFS and neuroischemic form of DFS, where the frequency of anaerobic bacteria isolation with chronic inflammatory process was observed in 68.4% and with acute inflammation—in 98.9%. The bacterial content after the surgical debridement of purulent focus in neuropathic form of DFS in the course of comprehensive treatment during 2–3 days was reduced from 10.8 ± 0.2 to 3.8 ± 0.3. The relevance of the use of high-potent antiseptics such as Octenisept and Decasan in the treatment of purulent lesions of foot should be pointed out. When using Decasan antiseptic for the treatment of foot phlegmon, rinsing with Decasan solution + pad application during 20 min and applying gel into a wound, the bacterial content was significantly reduced: upon the initial data of CFU concentration 10.8 ± 0.32 in 5 days—7.3 ± 0.24, in 10 days—5.9 ± 0.51, in 15 days—4.8 ± 0.72, in 21 days—3.8 ± 0.36, while when using Decasan solution in 5 days—9.7 ± 0.58, in 10 days—8.4 ± 0.73, in 15 days—7.3 ± 0.48, in 21 days—6.2 ± 0.61. The clinical course and prognosis were more favourable when using Decasan.

Conclusions: The microbial landscape of PNC in DFS is distinguished by different multistrain composition and high antibiotic resistance. The antiseptic for local application Decasan is an important element of antimicrobial therapy of PNC in DFS.

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Antibiotic resistance in sepsis patients in a Romanian hospital

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Introduction: Early antibiotic therapy in septic patients is usually empirical and it is based on several risk factors including the site of the infection, presumed etiology and local resistance pattern. Romania is currently listed as having one of the highest antibiotic resistance rates in Europe, leading to a high use of carbapenems and vancomycin as a first-choice regimen. Improving the knowledge about the local antibiotic resistance could help improve the current empirical regimens.

Objectives: Study on antimicrobial resistance in patients with sepsis from a Romanian hospital

Methods: A retrospective observational study was conducted in adult patients admitted with sepsis during Jan 2017- June 2017 in one of the wards from Matei Bals Institute of Infectious Diseases, Bucharest, Romania. The diagnosis of sepsis was established based on SOFA criteria (Sepsis 3, 2016) and/or Sepsis 2 criteria (2001). Isolates were identified and antibiotic sensitivities were performed using standard microbiological procedures.

Results: A total of 140 microbiological samples were analysed. Half of the blood cultures were positive (58.33%), most of cases for Gram-negative germs (58%) followed by Gram-positives (35.71%). Importantly most *K. pneumoniae* and *E. coli* strains were ESBL producing bacteria (60 and 40% respectively). 28.37% of the Gram-negative strains produced carbapenemases as well (80% in *A. baumanni,* 50% in *P. aeruginosa* and 36.84% in *K. pneumoniae* cases). On the other hand 94.73% of *S. aureus* strains were MRSA vancomycin sensitive. 72% of all strains were MDR. We recorded an important increase in colymycin-resistant strains (71.62%), fluoroquinolones (56.64%) and gentamycin (49.12%) strains, but not towards tygecycline yet (8.16%).

The Gram-negative strains were observed in 82.85% of all deceased patients (ESBL positive strains 32% and carbapenemase producers 17.14%). Most frequent Gram-negative isolates were *E. coli* (25.71%), *P. aeruginosa* (17.14%), *K. pneumoniae* and *A. baumanii* (11.42%). The Gram-positive strains were observed in 31.42% of deceased patients with a death rate due to MRSA of 17.14%. Polymicrobial infections were recorded in 31.42% of the patients with an unfavourable outcome.

Conclusions: In conclusion the use of vancomycin in settings with a high incidence of MRSA remains a good choice. On the other hand the increasing rates of ESBL and carbapenemase- producing Gram negatives prompt the need for alternative regimens, such as tigecycline. Nevertheless, the Pseudomonas and Acinetobacter strains continue to have limited treatment options and a poor prognosis in high-resistance areas.

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Infection 2017

International registry on the use of the CytoSorb®-adsorber in ICU patients (NCT02312024)—preliminary results

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Introduction: Clinical registries are valuable tools for assessing the effects of medical applications under real-life conditions.
Objectives: The aim of this registry is to record the use of CytoSorb® adsorber device in critically ill patients in as many cases as possible.
Methods: The objectives of the registry (http://www.cytosorb-registry.org/) are collection of data on a broad scale, centralized, structured and comprehensive documentation, and controlled data exchange. The registry records all relevant information in the course of product use, e.g. diagnosis, comorbidities, course of the condition, treatment, concomitant medication, clinical laboratory parameters and outcome (ClinicalTrials.gov Identifier: NCT02312024). Primary endpoint is in-hospital mortality as compared to the mortality predicted by the APACHE-II and SAPS-II-Score, respectively.
Results: As of June 30, 2017, 142 centers from 24 countries were participating in the registry and 345 patients were documented. Data available from the start of the registry on May 18, 2015 to November 24, 2016 were analyzed. At this time point 122 centers from 22 countries participated in the registry, of whom 20 centers from four countries provided data for a total of 198 patients. Mean age was 60.3 ± 15.1 years, 135 (68.2%) were male. 192 (97.0%) had one to 5 Cytosorb adsorber applications. Sepsis/septic shock was the most common indication for CytoSorb treatment (135 patients). Mean APACHE-II score in this group was 33.1 ± 8.4 [range 15–52]) with a predicted risk of death of 78%, whereas the observed mortality was 65%. There were no significant decreases in the SOFA scores after treatment (17.2 ± 4.8 [3–24]). However, IL-6 levels were markedly reduced after treatment (median 5000 pg/ml before and 289 pg/ml after treatment, respectively).
Conclusions: This third interim report demonstrates the feasibility of the registry with excellent data quality and completeness from twenty study centers. The results must be interpreted with caution, since the numbers are still small, however the disease severity is remarkably high and suggests that adsorber treatment might be used as an ultimate treatment in life-threatening situations. There were no device-associated side effects.

Longterm Outcome

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Infection 2017

The malicious economics of sepsis: the long-term outcome is significantly worse than immediate financial burden

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Introduction: It is generally accepted that sepsis is an acute emergency condition. Multiple reports warn about well-proved and possible long-term morbidity and mortality. Other studies report no significant changes in long-term health status between post-septic and non-septic individuals. However, its long-term consequences remain unidentified and unappreciated. While financial burden of sepsis is extremely heavy, the total cost of mortalities, treatment, and disabilities throughout the life is not yet estimated.
Objectives: The aim of the study is to determine the accumulative financial effect of sepsis and its consequences during long-term observation.
Methods: The study is based on local register of 836 septic patients (mean age 53.61 ± 9.14 yrs) mutually created by several General Surgery clinics in West Ukraine. Medical problems associated with sepsis and included into the study covered cumulative acute and post-acute mortality as well as long-term mortality; morbidities and complications were selected based on intensive database search (Cochrane, Embase, Medline, Web of Science, and Google Scholar). Primarily unrelated medical conditions but with sepsis-associated complications like trauma complicated by SIRS/MODS were included only from the time of complication occurrence. Chronic disease expenses present at the time of initial sepsis were deducted. All figures converted to 'conditional credits' to avoid bias of rates' changes and inflation. Initial spending was taken as 100%. Calculations of financial expenses related to sepsis were based on the 'WHO Guide to identifying the economic consequences of disease and injury'.
Results: The most common health problems after sepsis were mental (cognitive, stroke, extreme weakness and fatigue, general body pains or aches, difficulty moving around and/or sleeping), cardio-vascular (heart failure, peripheral vascular, arrhythmias), infections, immunosuppression and organ failure. Accumulated 10 yrs mortality after sepsis due to sepsis-related causes reached 16.03% (acute mortalities unrelated e.g. traumatic injuries excluded). General disease associated financial during 10 yrs of observation rose almost sextuple, reaching dramatic 586.9 ± 48.16%. Among most common post-septic medical conditions consuming financial resources were different infectious/impaired immunity complications.
Conclusions: Some of the long-lasting effects of sepsis are obvious, such as missing limbs or organ dysfunctions, like kidney failure or cardiovascular problems. Other after effects of sepsis are less obvious,

such as memory loss or the inability to do simple arithmetic. The after care for septic patients is needed not only in the hospital environment, but at home as part of community care. This makes sepsis a long-term financial burden for both families and communities, backing the idea of early beginning of post-sepsis rehabilitation and secondary prophylaxis.

026
Infection 2017

The Mid-German Sepsis Cohort (MSC)—background, study design and status quo

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Introduction: Patients with sepsis experience life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al. 2016). Recent estimates of highly-variable population incident rates from high-income countries ranged between 148 and 288 hospital-treated sepsis cases per 100,000 person-years depending on disease severity (Fleischmann et al. 2016).
For survivors, however, sepsis is not over after the intensive care therapy and a wide range of sepsis sequelae has been reported. Sepsis has therefore been called a "hidden" healthcare disaster (Angus 2010). While these adverse and long-lasting post-discharge outcomes are increasingly acknowledged, we lack a comprehensive assessment of the nature, extent, and impact of sepsis sequelae. A systematic review (Winters et al., 2010) that summarized the available evidence until 02/2009 demonstrated that studies mainly focus on long-term mortality and decreased quality of life. When we recently updated this systematic review, we came to the same conclusions.
Objectives: We designed the Mid-German Sepsis Cohort (MSC) as a patient cohort to better and more completely quantify mid- and long-term functional disabilities resulting from sepsis (Scherag et al. 2017).
Methods: We include all ICU-treated patients with (severe) sepsis. Recruitment is based on ICUs of six large hospitals in Thuringia, Saxony-Anhalt and Saxony. After the initiation in 03/2016, we aim at including 3000 patients during a period of 3 years to obtain a sufficiently large sample of survivors discharged from the ICU to assess the nature, extent, and impact as well as the time course of physical, mental, and emotional outcomes over a period of up to 5 years.
Results: In addition to the medical background and study design information, we provide an update on the number of already included patients.
Conclusions: After recruitment is finished, we can expect the first results of the MSC.

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#MSC Investigators: The authors and Bauer M, Kortgen A (all Jena University Hospital), Schreiber T, Hüter L (both Zentralklinik Bad Berka), Oehmichen F (Bavaria Clinic Kreischa), Meier-Hellmann A (Helios Clinic Erfurt), Petros S, Bercker S, Stehr S (all Universitätsklinikum Leipzig), Bucher M, Nuding S, Frantz S (all Universitätsklinikum Halle (Saale)).

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Familial coping in the post discharge process after surviving sepsis

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Introduction: Currently, little is known about the post discharge process of survived sepsis. Recent studies have shown that survivors commonly suffer from chronical diseases such as posttraumatic stress disorder, and experience a reduction in health-related quality of life. Also it is known that survivors often need help in coping with their everyday lives and are dependent on the support of family members. But at this stage, it is unclear how the survivors and family members cope exactly. Questions that arise are: What post discharge challenges do they have, what strategies do they use and which of these strategies are successful to recover and return to everyday life?
Objectives: To examine and describe challenges and coping strategies in the post discharge process in one family, in which one person survived a sepsis.
Methods: The method used is the "case-reconstructive family research", which encompasses serval methodological approaches, such as grounded theory and phenomenology. The data collection includes interviews, genograms and observations. Hermeneutic analysis instruments are used for data analysis.
Results: This study investigates the case of Mr. and Mrs. Schnell as a family. Mr. Schnell survived a sepsis at the age of 59. After Mr. Schnell came home, he still depended on medical devices and was confined to bed. Mrs. Schnell described the homecoming moment as

the biggest challenge, because she did not know what to do, to give her husband right medical support. Additionally, she described that no physician was available and no information was given about how to deal with this situation. To cope with this stressful situation, Mr. and Mrs. Schnell designed a nurse-patient relationship in which Mrs. Schnell became the absolute authority, even towards medical and therapeutic professions. Mr. Schnell reported that it was tough to be immobile, even more because the ability to walk had been an imperative of his identity as a handicapped person. He worked very hard to gain physical strength to keep the hope to walk again. These strategies seemed successful, because Mr. and Mrs. Schnell said that they are satisfied today. Conditions that contributed to their successful coping might be Mr. Schnell's resilient identity as a handicapped person and Mrs. Schnell's past as a nurse.

Conclusions: One major challenge for sepsis survivors and their families is the homecoming moment. This event should be supported by family members and professionals.

045
Infection 2017

Superior accuracy of mid-regional proadrenomedullin over C reactive protein, procalcitonin and lactate for ICU mortality prediction in septic patients

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Introduction: Recently, studies of the potential usefulness of the mid-regional proadrenomedullin (MR-proADM) as mortality predictor in sepsis has provided interesting results [1, 2, 3, 4].

Objectives: The aim of our study was to evaluate the accuracy of MR-proADM to predict ICU mortality in septic patients compared with other more frequently used biomarkers, procalcitonin (PCT), C reactive protein (CRP) and lactate, and APACHE II and SOFA scores.

Methods: This was a single-centre, prospective, observational, cohort, blind study, enrolling septic patients admitted to the ICU of a teaching tertiary hospital between November 2016 and April 2017. The Institutional Review Board approved the study. Blood samples for the determination of MR-proADM were taken within the first 18 h of ICU admission, after obtaining informed consent. The rest of biomarkers were analysed from the first blood sample taken upon ICU admission as well as the APACHE II and SOFA scores. The accuracy of the biomarkers and risk scores for ICU mortality was evaluated by calculating the area under the receiver operating characteristic (AUROC) curve. Survival was assessed by Kaplan Meier and COX regression analysis.

Results: Thirty-three consecutive patients with sepsis (20.2%) or septic shock (78.8%) were enrolled. The ICU mortality was 27.3%. The mean age was 69.9 years (standard deviation [SD], 11.6) and 66.7% of patients were male. MR-proADM and lactate plasmatic levels were both significantly higher in non-survivors: 16.89 nmol/L (8.86–27.44) versus 3.83 nmol/L (1.04–8.86) ($p < 0.001$) and 5.56 mmol/L (1.50–10.50) versus 2.35 mmol/L (0.50-9.10) ($p = 0.007$), respectively. Non-surviving patients also had both a higher APACHE II score (31 [24–35] vs 20.5 [10–42]) ($p = 0.004$) and SOFA score (11 [8–14] vs 8 [1–15]) ($p = 0.032$). Although plasmatic levels of CRP and PCT were higher in the patients who died, this effect was not statistically significant (Table 1).

Conclusions: A unique determination of MR-proADM plasmatic levels in septic patients measured within the first 18 h of admission in the ICU has better accuracy for ICU mortality prediction than CRP, PCT, lactate or APACHE II and SOFA scores.

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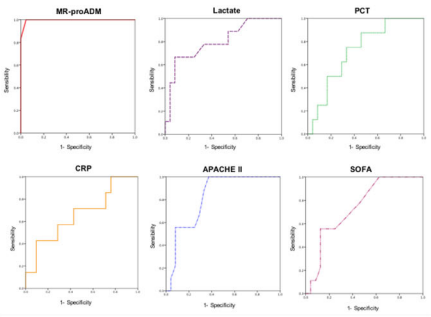

Figure 1. ROC curves for biomarkers and severity scores with respect to ICU mortality

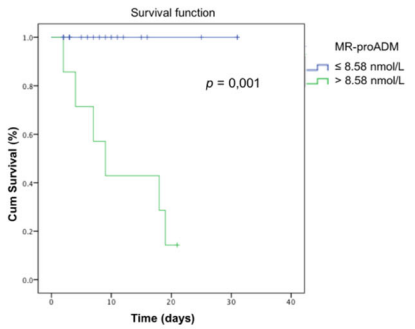

Figure 2. Kaplan-Meier curves for a MR-proADM cut-off value of 8.58 nmol/L

Table 1. Characteristics of the patients

	Survivors (n = 24)	Non-survivors (n = 9)	Total (n = 33)	p
Demographics				
· Patients (n, %)	24 (72.7)	9 (27.3)	33 (100)	
· Male (n, %)	17 (70.8)	5 (55.6)	22 (66.7)	0.438
· Age, years (mean, SD)	69.3 (12.5)	71.8 (9.3)	69.9 (11.6)	0.586
· BMI, kg/m² (mean, SD)	25.6 (7.1)	25.1 (4.4)	25.5 (6.4)	0.848
Origin				
· Emergency department (n, %)	7 (29.2)	3 (33.3)	10 (30.3)	
· Ward (n, %)	4 (16.7)	3 (33.3)	7 (21.2)	
· Surgery (n, %)	12 (50)	3 (33.3)	15 (45.5)	0.644
· Other hospital (n, %)	1 (4.2)	-	1 (3)	
Comorbidities				
· High blood pressure (n, %)	12 (50)	8 (88.9)	20 (60.6)	0.056
· Cardiovascular disease (n, %)	9 (37.5)	4 (44.4)	13 (39.4)	1.000
· COPD (n, %)	6 (25)	1 (11.1)	7 (21.2)	0.642
· Chronic kidney disease (n, %)	5 (20.8)	1 (11.1)	6 (18.2)	1.000
· Chronic liver disease (n, %)	3 (12.5)	0 (0)	3 (9.1)	0.545
· Diabetes (n, %)	5 (20.8)	5 (55.6)	10 (30.3)	0.090
· Cancer (n, %)	6 (25)	8 (88.9)	14 (42.4)	0.002
· Immunosuppression (n, %)	3 (12.5)	1 (11.1)	4 (12.1)	1.000
Infectious origin				
· Respiratory (n, %)	11 (45.8)	2 (22.2)	13 (39.4)	
· Abdominal (n, %)	11 (45.8)	5 (55.6)	16 (48.5)	
· Urinary (n, %)	1 (4.2)	1 (11.1)	2 (6.1)	0.329
· Septic arthritis (n, %)	1 (4.2)	-	1 (3)	
· Soft tissues (n, %)	-	1 (11.1)	1 (3)	
Clinical variables				
· MAP, mmHg (median, IQR)	65.8 (45-80)	52 (42-59)	59 (54.5-67)	< 0.001
· Heart rate, bpm (median, IQR)	113 (68-147)	148 (115-170)	120 (104-134.5)	< 0.001
· Temperature, °C (median, IQR)	37.1 (35-41)	37.8 (35.1-38.5)	37.2 (35.8-38.3)	0.858
· Respiratory rate, rpm (median, IQR)	20 (6-40)	26 (16-38)	23 (15-29)	0.036
· Glasgow score, points (median, IQR)	14 (6-15)	13 (11-15)	14 (12-15)	0.890
Laboratory variables				
· MR-proADM, nmol/L (median, IQR)	3.83 (1.04-8.86)	16.89 (8.86-27.44)	4.98 (3.22-8.72)	< 0.001
· Lactato, mmol/L (median, IQR)	2.35 (0.50-9.10)	5.56 (1.50-10.50)	3.20 (1.50-4.25)	0.007
· PCT, ng/mL (median, IQR)	3.90 (0.20-118.70)	29.79 (2.40-74.20)	6.30 (1.80-35.05)	0.064
· CRP, mg/dL (median, IQR)	16.30 (1.65-43.70)	25.43 (6.88-49.70)	17.35 (10.1-26.2)	0.228
· White blood cells, x10³/L (median, IQR)	14.40 (0,32-35,00)	9.40 (0.10-23.70)	11.9 (4.1-19.1)	0.207
· Platelets, x10³/L (median, IQR)	185.50 (58-401)	125 (10-535)	173 (107.5-310)	0.414
· Creatinine, mg/dL (median, IQR)	1.13 (0.35-4.10)	2.30 (0.21-6.09)	1.6 (0.9-2.2)	0.032
Severity scores				
· APACHE II, points (median, IQR)	20.5 (10-42)	31 (24-35)	24 (17-30)	0.004
· SOFA, points (median, IQR)	8 (1-15)	11 (8-14)	9 (7-10,5)	0.032
Other				
· Septic shock upon ICU admission (n, %)	17 (70.8)	9 (100)	26 (78.8)	0.149
· ICU stay, days (median, IQR)	7.5 (2-31)	15 (2-46)	9 (3-17)	0.193
· ICU mortality (n, %)			9 (27.3)	
· 28 day mortality (n, %)			14 (42.4)	

BMI body mass index, COPD chronic obstructive pulmonary disease, TBC tuberculosis, PCR polymerase chain reaction, IQR interquartilic range, IMV invasive mechanical ventilation, SOFA sequential [sepsis related] organ failure assessment

Table 2. AUROC analysis for ICU mortality prediction

Biomarker	AUC (95% CI)	p	Cutt-off point	Se %	Sp %	PPV %	NPV %
MR-proADM	0.99 (0.98-1.00)	< 0.001	8.58	100	95.5	85.7	100
Lactate	0.80 (0.63-0.98)	0.009	3.35	77.8	66.7	46.7	88.9
PCT	0.72 (0.54-0.91)	0.061	4.10	87.5	458	38.9	92.9
CRP	0.66 (0.42-0.90)	0.212	17.35	71.4	57.1	36.8	85.7
APACHE II	0.82 (0.68-0.96)	0.005	23.00	100	62.5	50	100
SOFA	0.74 (0.57-0.92)	0.034	8.50	77.8	54.2	38.9	86.7

AUC Area Under The Curve, *Se* sensibility, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Table 3. Univariate and multivariate COX regression analysis for ICU mortality prediction

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
MR-proADM	1.12 (1.03-1.22)	0.012	1.72 (1.02-2.92)	0.043
Lactate	1.45 (1.06-2.00)	0.022	1.37 (0.88-2.14)	0.160
PCT	1.01 (0.98-1.03)	0.677	-	-
CRP	1.03 (0.97-1.10)	0.311	-	-
APACHE II	1.18 (1.04-1.34)	0.011	2.95 (1.01-8.59)	0.048
SOFA	1.28 (0.97-1.69)	0.083	0.09 (0.01-1.02)	0.052

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Infection 2017

Internet-based cognitive-behavioural writing therapy for reducing post-traumatic stress after intensive care for sepsis in patients and their spouses (REPAIR): results of two pilot cases

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Introduction: As consequence of sepsis and intensive care unit treatment, a considerable number of patients and their spouses develop symptoms of posttraumatic stress disorder (PTSD). Internet-based cognitive-behavioural writing therapy (IB-CBWT) has proven to be an effective treatment option for PTSD in various studies. This therapeutic approach may allow to meet the specific needs of sepsis survivors and their spouses and to overcome treatment barriers. In a randomized-controlled trial (REPAIR) we currently examine the feasibility, acceptance, safety, and efficacy of IB-CBWT for PTSD in patients and their spouses after intensive care for sepsis.
Objectives: To get first information about feasibility, acceptance, safety and efficacy, we aim to analyse the results of two pilot patient-partner-dyads who received IB-CBWT.
Methods: In the REPAIR trial participating patient-partner-dyads are assigned by random either to a treatment or a wait-list (WL) control group. The treatment group receives IB-CBWT for PTSD. If both the patient and the partner show increased PTSD symptom scores measured by PTSD Checklist for DSM-5 (PCL-5), both obtain IB-CBWT. If only one of them is affected, the partner is actively involved in the participant's IB-CBWT. IB-CBWT is guided by an experienced therapist and consists of two writing assignments per week over a period of 5 weeks. After completion of each assignment, the participants receive individual feedback from the therapist. Participants assigned to WL control group obtain treatment after a 5-week waiting period. The primary outcome is PTSD symptom severity in self-rated PCL-5 at the end of treatment and waiting time, respectively. Two patient-partner-dyads were treated as pilot cases before the main study has started. After completion of the intervention they were interviewed and asked for their experiences and evaluation regarding their treatment participation.
Results: According to the initial PCL-5 scores, both the patient and the partner of the first pilot dyad received IB-CBWT. In the second pilot dyad only the sepsis patient was treated with IB-CBWT. We obtained positive evidence regarding feasibility, acceptance and safety of IB-CBWT. Neither dropouts nor adverse events occurred. Furthermore, participants reported high satisfaction with the treatment and the study modalities. According to their statements, participation in the REPAIR trial was beneficial for both, the sepsis patients as well as their partners.
Conclusions: The analysis of pilot cases is an important instrument in psychotherapy research, especially with respect to the development of therapy concepts. Because of the small sample size generalizability of the results is limited. Nevertheless, results of the two pilot cases reveal that IB-CBWT is a promising approach to treat patients and their spouses suffering from PTSD symptoms after intensive care for sepsis. The case results have to be replicated in the REPAIR trial's total sample.

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Infection 2017

Sepsis induces long-term changes in the transcriptome and epigenome of naïve bone marrow monocytes

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Introduction: The late phase of sepsis is often associated with a so-called "sepsis-induced immunosuppression". This state is characterized by a persisting hypo-responsiveness of the host's immune system (also "compensatory anti-inflammatory response syndrome") to counteract the pronounced initial pro-inflammatory response ("systemic inflammatory response syndrome"). This overall immunosuppressive state renders the host vulnerable for a persisting or the occurrence of secondary, often opportunistic infections. As a result, this condition increases mortality both within and even after discharge from the hospital, raising it an important aspect of the follow-up treatment of sepsis survivors.
Objectives: The mechanisms underlying the immunosuppressive phenotype are yet unknown, but with respect to the longevity of it, the involvement of epigenetic alterations in DNA methylation or histone modifications of immune cells seem obvious. Our project aims to unravel these underlying molecular mechanisms.
Methods: Polymicrobial abdominal sepsis was induced in 12 weeks old C57BL7/6 mice using the cecal ligation and puncture (CLP) model. Corresponding control animals only received a laparotomy without intestinal perforation. Three months after insult, surviving mice were euthanized, and Cd11b+ Ly6C+ monocytes were isolated from bone marrow by magnetic activated cell sorting. Subsequently, genome-wide distribution of the active histone mark H3K4me3 was analyzed by ChIP-Seq and global gene expression by RNA-Seq. Also, immune cell composition and functionality in blood, spleen, and peritoneum were assessed by flow cytometry and ex vivo stimulation.
Results: Principal component analysis of global gene expression of naïve bone-marrow monocytes revealed a sustained deregulation of certain genes after CLP conditions: 75 genes were differentially expressed, with 2 down- and 73 genes up-regulated genes. Furthermore, an increase of H3K4me3 was observed in 77 promoter regions of post-septic naïve monocytes. No correlation between changes in H3K4me3 and altered gene expression could be determined. Furthermore, a robust change of immune cell abundance, especially of the lymphoid lineage, in spleen and peritoneum was obvious.
Conclusions: Our results prove the remains of transcriptomic scars in naïve bone marrow monocytes even months after the insult, potentially indicating an ab initio altered functional state of naïve monocytes. Interestingly, the increase in gene expression was not associated with histone alterations, leaving the question of the involved regulatory tier open for further research.

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Infection 2017

Influence of paternal sepsis on behavior and stress response in offspring

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Introduction: The hypothalamus-pituitary-adrenal (HPA) axis is a central neuroendocrine circuit in the response of the body to stressful situations like overwhelming immunological reactions during sepsis. It is activated e.g. by pro-inflammatory cytokines, leading ultimately to the release of steroids like e.g. cortisol or its murine paralogue corticosterone from the adrenal glands. Several studies reported the trans- and intergenerational heredity of parental adaptions to environmental exposures onto the progeny. Especially, parental distress has been proven to result in abnormal stress reactions and diminished HPA axis responsiveness in descendants.
Objectives: We aimed to evaluate if also paternal sepsis induces intergenerational changes of behavior and stress response in their offspring.
Methods: C57BL7/6 male mice (12 weeks) were subjected to cecal ligation and puncture (CLP), thereby inducing a polymicrobial peritonitis. Control animals did only receive a laparotomy without intestinal perforation. Six weeks after insult, each surviving male was bred with two healthy females. The resulting offspring were grown up to 15 weeks before behavioral assessment. All mice underwent a test sequence in open field, elevated O-maze and T-maze (24 h rest between each). To assess the acute stress reaction, animals were immobilized for 15 min and euthanized. Subsequently, the concentration of the stress-related hormones corticosterone, ACTH and testosterone were determined in the blood plasma. Furthermore, gene expression analysis of molecular components of the HPA axis organs was performed by qPCR.
Results: Male offspring of post-septic fathers showed a mild increase in exploratory behavior, observed by increased moving time and distance in the open field test. No differences in behavior could be found in the O-Maze test. In line, males (and not female offspring) approached a foreign object introduced during the open field test more often than the corresponding controls. Interestingly, female animals were more exploratory, but also insecure during the elevated O-maze, what resulted in several falls. After acute immobilization stress, male CLP descendants showed no changes in the plasma ACTH and corticosterone, whereas a higher release of ACTH could be observed in female sham descendants. Regarding basal gene expression, an elevated expression of the CRH gene in CLP progenies of both genders was observed. CRHR2 gene was solely reduced in male CLP descendants. In contrast, POMC gene expression was only elevated in CLP female offspring.
Conclusions: We can provide evidence for intergenerational inherited alterations in molecular and behavioral aspects of sepsis progenies. Remarkably, the phenotype seems to be sex-dependent, with male CLP descendants to be more stress-resistant and less anxious than control descendants.

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Infection 2017

Quantitative virtual infection modeling of sepsis

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Introduction: A systemic inflammatory response caused by a severe infection is called sepsis and can result into organ dysfunction that may lead to death. The immune response is a highly complex system involving non-linear mechanisms. Because of the high complexity of the biological data it is often difficult to unravel the mechanisms, which are important to predict the outcome of the disease or to suggest therapeutic options. A systems biology approach consisting of a combination of mathematical models and quantitative biological data promises deeper insights into the processes of the biological

system. Here we investigate an infection model of a liver organoid. The liver is a critical organ for host survival in the context of sepsis and plays a central role in metabolic and immunological homeostasis. Therefore, it is a key player in the host response against infections, as well as in damage and repair.
Objectives: By deriving a virtual infection model of the liver organoid and comparing the model to experimental data, we can investigate potential immune response mechanisms and validate or neglect these hypotheses. Furthermore we can predict possible outcomes of an infection and propose new immune response mechanisms that can be verified in experiment.
Methods: The virtual infection model is based on a set of biological reactions describing the interplay of invading pathogens, immune cells like macrophages and parenchymal cells like hepatocytes. The model describes the temporal evolution of the cell concentrations and is fitted to data of a liver organoid to determine the parameters of the model.
Results: The virtual infection model can predict different outcomes of the disease. Depending on the initial conditions and the rates of the system, possible outcomes of the disease are clearance of the pathogens and immune homeostasis, clearance of the pathogens associated with overwhelming inflammation or persistent pathogen populations. By varying critical parameters, transitions between the different outcomes can be observed.
Conclusions: The virtual infection model of a liver organoid gives the opportunity to investigate the relative importance of various immune response mechanisms. By identifying critical parameters of the system, we will be able to propose further experiments and get deeper insights into the processes of sepsis.

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Infection 2017

Sepsis long-term mortality assessed by the Jena Sepsis Registry

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Introduction: Most existing sepsis registries are restricted to hospital data. There is still few population-based data on long-term mortality of survivors of sepsis and septic shock.
Objectives: To describe long-term mortality of sepsis and septic shock in association to clinical and other factors.
Methods: All ICU patients of the Jena University Hospital between 2011 and 2015 were screened for severe sepsis or septic shock. Clinical, microbiological, sociodemographic and process-of-care data was extracted from patient ICU records. Follow-up data on mortality was collected at the patients' primary care providers at 6, 12, 24, 36 and 48 months after diagnosis. Primary outcome is all-cause mortality at 6 months.
Results: N = 1976 patients were included meeting criteria for sepsis or septic shock. Of these, 44.7% (N = 883) died in hospital. Six month after diagnosis, mortality increased to 58.5% (N = 1156). N = 55 patients (2.8%) were lost to follow up. Six-month mortality was significantly associated with age, APACHE II score, recent surgical history, pneumonia, history of liver cirrhosis and nosocomial acquired source of infection. Over time, mortality increased to 74.2% (N = 288) at 48 months referring to N = 388 patients included in 2011.
Conclusions: The Jena Sepsis Registry provides observational long-term data on mortality of sepsis and septic shock up to 48 months after diagnosis. Even though validity is limited due to the monocentric design, the registry demonstrates feasibility indicated by high enrolment and follow up rates.

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Infection 2017

Long term effect of a sepsis aftercare intervention

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Introduction: Sepsis survivors face mental and physical long-term sequelae. A structured sepsis aftercare intervention has been implemented in primary care. The intervention group showed indications of better functional outcomes than the control group at 6 and 12 months. It is unknown if this result persists.
Objectives: To examine the functional long term outcomes of a primary care-based case management intervention in sepsis aftercare
Methods: A 24-month follow-up of the SMOOTH study [1], a randomized clinical trial, was performed 12 months after the 1-year intervention. Patients with severe sepsis or septic shock treated at one of nine ICU study centers were randomized to intervention and control group. The intervention included training of patients and treating primary care physicians (PCP), case management provided by trained nurses and clinical decision support for PCPs by consulting physicians. Usual care was provided by PCPs in the control group. Data were collected by telephone interviews at 6, 12 and 24 month using validated questionnaires assessing physical and musculoskeletal functioning, activities of daily living among 28 other outcomes.
Results: 143 and 148 patients were randomized to the intervention and control group, respectively. 186 of 291 (63.9%) patients participated 24-month follow up. Differences in functional outcomes between intervention and control group at 6 and 12 months, as has been reported before [1] did not sustain at 24 months.
Conclusions: By increased physical function across both groups at 24 month after ICU discharge, there was possibly no potential for functional improvement by the intervention. In addition, possible improvements at 6 and 12 months in the intervention group did not sustain at 12 months after intervention termination.
Reference: 1: Schmidt, Konrad, et al. "Effect of a Primary Care Management Intervention on Mental Health–Related Quality of Life Among Survivors of Sepsis: A Randomized Clinical Trial." Jama 315.24 (2016): 2703-2711.
Acknowledgement: SMOOTH Study Group

Sepsis Prevention

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Infection 2017

Quality-management for sepsis treatment in African anaesthesia through training of non-doctor anaesthetists

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Introduction: Most anaesthesia and ICU-care for septic patients in Malawi is given through Bachelors of Science and clinical officers in Anaesthesia (BACOS). Quality-management needs to know the type of their training, which chances for careers might be in reach and the challenges they face.
Objectives: To investigate on the ground standard training, further education and problems of quality-management for non-doctor anaesthetists.
Methods: We evaluated the situation through semistructured interviews and questionnaires in the Ministry, the University of Malawi and the relevant hospitals and compared informations with 10 years experience in the country and the literature.
Results: Different types of non-physician anaesthetists dealing with sepsis in Malawi were found. The anaesthetic clinical officer (around 120 in 4 central and more than 30 district or rural hospitals), their trainees (32) and interns (16) and the Bachelors of Sciences in Anaesthesia and Intensive Care (17) and ist trainees (12). Only very occasionally we found a nurse or another profession providing anaesthesia for sepsis. We found significant improvement in their numbers of almost 100% from´07 (100) to´17 (197) due to intense measures in training and the inauguration of a BSc. Nevertheless numbers are far behind staff establishments (<25%). In May 2017 all schools and training institutions for BACOS are working. Relations of professional groups dealing with sepsis are complex and face challenges (age up to 75, working despite a major stroke, on-call numbers, low salary, own HIV or malaria infection, low CME resources, <1% study medicine, bad relations to ICU nurses, low standing in medical community).
Conclusions: Training of non-doctors (for quantity and quality) is crucial to reach the traditional development goals (sustainability, empowerment, capacity building) in sepsis treatment too.

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Infection 2017

Nationwide trends in infection and sepsis incidence and mortality in Germany

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Introduction: Over the last decade, sepsis incidence rates were found to increase in multiple countries by up to 5.7% annually (1). Demographic change and an increase of invasive or immunosuppressive measures may be important contributors, as well as an increased sepsis awareness. The impact of changes in infectious disease incidences is unknown in Germany.
Objectives: To assess trends in infection and sepsis incidence and mortality between 2010 and 2014 based on administrative data in Germany.
Methods: Patients with (1) infection, (2) infection and organ dysfunction, (3) infection and intensive care/organ support as well as (4) sepsis were identified in a nation-wide database of hospital discharge data (DRG statistics) between 2010 and 2014 using ICD-10 codes used as primary and secondary discharge diagnoses and procedural OPS codes. Sepsis was defined using explicit sepsis codes microbiological and/or clinical sepsis codes). Severe sepsis and septic shock were defined as ICD-10 R65.1 and R57.2, respectively. Organ dysfunctions were identified by 27 ICD codes for cardiovascular, respirator, central nervous, renal, metabolic, hematologic and hepatic organ failure.
Results: In 2014, 4,035,853 patients with infectious diseases were treated in German hospitals, of which 26% had at least one organ dysfunction and 12% required intensive care, renal replacement therapy or mechanical ventilation (Tab. 1). 299,464 patients had sepsis, including 123,295 patients with severe sepsis and septic shock. Hospital mortality was 5.6% in infection patients and increased if an organ dysfunction or explicitly coded sepsis was present to 16.6 and 22.7%, respectively). Between 2010 and 2014, the age- and sex-adjusted incidence rate of hospitalized patients with infectious diseases increased by a mean of 1.6% annually and the number of those with organ failure or sepsis increased, too. The most substantial rise was found in patients with severe sepsis (mean +7.6% annually). Mortality declined or remained relatively stable. However, the absolute number of deaths from infectious diseases and sepsis increased over time.
Conclusions: Although the number of hospitalized patients with infections is growing, the numbers of those with infections complicated by sepsis, organ dysfunction or requirement for intensive care is rising. In contrast to sepsis, the latter are less prone to coding incentives. These dynamics are independent from demographic changes and confirm that sepsis must become a key priority for health care authorities and health care providers on the national and international level as recently underlined by a WHO resolution on sepsis. Further research is needed to understand the underlying mechanisms of rising infection and sepsis rates in German hospitals.
Reference: Fleischmann et al. Hospital Incidence and Mortality Rates of Sepsis. Dtsch Arztebl Int. 2016 Mar 11;113(10):159-66.

	infection (all)		infection and organ dysfunction		infection and mech. ventilation/ICU/RRT *		sepsis (incl. severe sepsis and shock)		severe sepsis (incl. septic shock)	
	2010	2014	2010	2014	2010	2014	2010	2014	2010	2014
cases	3,691,241	4,035,853	770,258	1,049,239	417,175	486,818	229,214	299,464	87,973	123,295
incidence/100.000	4,515	4,802	942	1,226	510	574	280	352	108	144
deaths	221,098	233,589	147,849	176,829	92,583	103,824	61,068	68,977	42,084	51,575
mortality	6.0	5.6	19.2	16.6	22.2	21.0	26.6	22.7	47.8	41.5
mean annual increase in incidence rate 2010-2014	+1.6%		+6.9%		+3.0%		+5.9%		+7.5	

* patients with infection and mechanical ventilation/renal replacement therapy or ICU therapy

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Infection 2017

Incidence of respiratory infections and sepsis in Germany

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Introduction: Respiratory infections are a substantial health care burden all over the world. Especially in elderly or immunocompromised patients, infections may take a more complex progression and lead to a sepsis.
Objectives: The aim of this study is to examine the incidence of respiratory infections in general, and specifically the incidence of pneumonia and influenza, and to determine the extent of severe complications as sepsis in Germany.

Methods: We retrospectively analyzed hospital discharge data from the year 2014 by using the DRG (diagnosis related groups) statistics of the German Federal Statistical Office (FSO), which contains nearly complete data of all inpatient hospital treatments in Germany. The cases were identified among primary and secondary discharge diagnoses by ICD-10 codes: Influenza—J09, J10 or J11; pneumonia—J12–J19 or U69.00!; sepsis—at least one of 29 microbiological and clinical codes; severe sepsis—R65.1 or R57.2. We assessed incidences (total number of cases, cases per 100,000 population) and mortality and describe age, sex, comorbidity and treatment data of patients.

Results: In 2014, 1,345,848 patients were hospitalized due to respiratory infections, which made up 33.3% of all infectious disease cases treated in German hospitals. About 50% of the respiratory infections were coded as pneumonia, whereas only 0.4% of respiratory infections were coded as influenza. Patients with pneumonia had a relatively high mortality of 15.7% and patients with influenza had a relatively low mortality of 2.6% when compared to the overall mortality of respiratory infections of 9.6%. 104,154 (7.7%) of patients with respiratory infections had sepsis, most of which were patients with pneumonia progressing into sepsis (88,939 cases; 6.6%). Mortality of pneumonia and influenza increased to 33.7% and 23.3% respectively, when accompanied by sepsis. In total, 34.8% of all sepsis cases occurred with a respiratory infection. Incidences for both respiratory infections and pneumonia increased with age, while incidence for influenza was highest in children.

Conclusions: Results show considerable proportions of patients with sepsis among patients with respiratory infections and a higher risk with increasing age—especially for pneumonia. The incidence of hospital-treated influenza is relatively low and underestimates the true burden of disease, as most patients may be treated in the outpatient setting (see RKI influenza report 2014 [1]). The results point out that a reduction of respiratory infections, e.g. by increasing vaccine uptake in the population, may be one way to achieve a substantial decrease in sepsis incidence.

Reference: 1. Arbeitsgemeinschaft Influenza (2014). Bericht zur Epidemiologie der Influenza in Deutschland Saison 2013/14. Retrieved from: http://influenza.rki.de/Saisonberichte/2013.pdf

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